COBAS® TaqMan® HBV Test
For Use With The High Pure System

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® TaqMan® HBV Test	**HBV HPS**	48 Tests	P/N: 03577163 190
High Pure System Viral Nucleic Acid Kit		48 Tests	P/N: 03502295 001

INTENDED USE

The COBAS® TaqMan® HBV Test For Use With The High Pure System is an *in vitro* nucleic acid amplification test for the quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma (EDTA), using the High Pure System Viral Nucleic Acid Kit for manual specimen preparation and the COBAS® TaqMan® 48 Analyzer for automated amplification and detection. The test is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy. The assay can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. The results from the COBAS® TaqMan® HBV Test must be interpreted within the context of all relevant clinical and laboratory findings.

Assay performance characteristics have been established for individuals treated with adefovir dipivoxil. Assay performance for determining the state of HBV infection has not been established.

The COBAS® TaqMan® HBV Test is not intended for use as a screening test for blood or blood products for the presence of HBV or as a diagnostic test to confirm the presence of HBV infection.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B Virus is one of several viruses known to cause viral hepatitis. Over two billion people throughout the world have been infected with HBV and over 350 million of them are chronically infected carriers.[1] Chronic carriers are at high risk of long term complications of infection, including chronic hepatitis, cirrhosis and hepatocellular carcinoma.[2,3,4] Serologic markers are commonly used as diagnostic and/or prognostic indicators of acute or chronic HBV infection. The most common marker of HBV infection is the presence of HBV surface antigen (HBsAg). Although carriers may clear HBsAg and develop antibody to HBsAg, there appears to still be a risk of serious liver complications later in life.[5,6] HBV e antigen (HBeAg) is generally used as a secondary marker to indicate active HBV replication associated with progressive liver disease. Failure to clear HBeAg appears to increase the risk of end stage liver disease.[7,8] Variant strains of HBV can either produce HBeAg that is not detectable in serum or the strain can lose the ability to make HBeAg even when an active infection is present.[9] Therefore, using this marker to monitor disease progression may be of limited utility since disease progression may occur in both HBeAg positive and HBeAg negative patients with active viral replication.[10] Guidance regarding therapy may also differ between HBeAg positive and HBeAg negative patients, as do outcomes with therapy.[10] Differences between patients with HBeAg positive and HBeAg negative disease include lower levels of HBV DNA typically found in HBeAg negative patients, even in those infected with pre-Core variants.[44] Recommendations for monitoring patients differ between HBeAg positive and HBeAg negative patients.[44] The presence or absence of HBeAg has also been used to guide treatment decisions in patients with chronic HBV disease. Endpoints of therapy differ between HBeAg positive and HBeAg negative patients. For HBeAg positive patients, response to treatment includes HBeAg loss and/or HBeAg seroconversion, as well as normalization of ALT, loss of HBV DNA and improvement in histology.[44] If HBeAg loss is achieved, the likelihood of a durable response is high. For HBeAg negative patients, response is defined as normalization of ALT, loss of HBV DNA and histologic improvement.[44] In contrast to treatment of HBeAg positive patients, the likelihood of a durable response after treatment of HBeAg negative patients is low. The ability to detect HBV DNA in serum has been reported to have prognostic value for the outcome of acute and chronic HBV infections.[11,12,13,14] The methodology can allow the detection of HBV DNA after HBsAg clearance[15] or detection of HBV lacking serologic markers.[16] However, a relationship between serologic markers

and HBV DNA levels has not yet been established. Although the efficacy of antiviral therapy used to treat patients with HBV can also be assessed by serologic markers or by measurement of liver enzyme function, the most direct and reliable measurement of viral replication is thought to be quantitation of HBV viral DNA in serum or plasma.[13,17,18,19] A rapid and sustained drop in HBV DNA levels in patients receiving treatment with alpha-interferon, lamivudine, entecavir, telbivudine, and peginterferon \2a- has been shown to be associated with a favorable treatment outcome.[10,20,21,22,23,24] Monitoring of HBV DNA levels can predict the development of resistance to lamivudine.[25] Therefore, a quantitative test for the measurement of HBV DNA is a valuable tool that can be used in conjunction with other clinical and laboratory findings in the management of HBV infection.

PRINCIPLES OF THE PROCEDURE

The COBAS® TaqMan® HBV Test is based on two major processes: (1) manual specimen preparation to obtain HBV DNA; (2) automated PCR amplification of target DNA using HBV specific complementary primers, and detection of cleaved dual fluorescent dye-labeled oligonucleotide detection probes that permit quantitation of HBV target amplified product (amplicon) and HBV Quantitation Standard (QS) DNA, which is processed, amplified, and detected simultaneously with the specimen.

The Master Mix reagent contains primer pairs and probes specific for both HBV DNA and HBV QS DNA. The Master Mix has been developed to ensure equivalent quantitation of genotypes A through G of HBV. The detection of amplified DNA is performed using target-specific and QS-specific dual labeled oligonucleotide probes that permit independent identification of HBV amplicon and HBV QS amplicon.

The quantitation of HBV viral DNA is performed using the HBV QS. The HBV QS is a non-infectious, linearized, double stranded plasmid that contains the identical primer binding sites as the HBV DNA target and a unique probe binding region that allows HBV QS amplicon to be distinguished from HBV target amplicon. The HBV QS is incorporated into each individual specimen and control at a known copy number and is carried through the specimen preparation, PCR amplification and detection steps along with the HBV target. The COBAS® TaqMan® 48 Analyzer calculates the HBV DNA titer in the test specimen by comparing the HBV signal to the HBV QS signal for each specimen and control. The HBV QS compensates for effects of inhibition and controls for the preparation and amplification processes to allow the accurate quantitation of HBV DNA in each specimen.

Specimen Preparation

The COBAS® TaqMan® HBV Test processes plasma and serum specimens and isolates HBV DNA through a generic manual specimen preparation based on nucleic acid binding to glass fibers. The HBV virus particles are lysed by incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released HBV DNA from DNases in plasma and serum. A known number of HBV QS DNA molecules are introduced into each specimen along with the lysis reagent. Subsequently, isopropanol is added to the lysis mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the HBV DNA and HBV QS DNA are bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and eluted with an aqueous solution. The disposables allow for a parallel processing of 12 specimens or multiples thereof. The processed specimen, containing HBV DNA and HBV QS DNA, is added to the amplification/detection mixture. The HBV target DNA and the HBV QS DNA are then amplified and detected on the COBAS® TaqMan® 48 Analyzer using the amplification and detection reagents provided in the Test kit.

PCR Amplification

Target Selection

Selection of the target DNA sequence for HBV depends on identification of regions within the HBV genome that show maximum sequence conservation among all genotypes. Accordingly, the appropriate selection of the primers and probe is critical to the ability of the test to detect all clinically relevant genotypes of HBV. A region of the partly single-stranded circular DNA genome of HBV has been shown to have maximum conservation of DNA sequences among genotypes. The COBAS® TaqMan® HBV Test uses PCR amplification primers that define a sequence within the highly conserved pre-Core/Core region of the HBV genome.

Target Amplification

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which PCR amplification occurs. The Thermal Cycler in the COBAS® TaqMan® 48 Analyzer heats the reaction mixture to

denature the double stranded DNA and expose the specific primer target sequences on the HBV circular DNA genome and the HBV Quantitation Standard DNA. As the mixture cools, the primers anneal to the target DNA. The thermostable *Thermus specie* Z05 DNA polymerase (Z05) in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of thymidine), extends the annealed primers along the target template to produce a double-stranded DNA molecule termed an amplicon. The COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the region of the HBV genome between the primers; the entire HBV genome is not amplified.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® TaqMan® HBV Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine,[26] but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme, thus improving sensitivity and specificity. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme is inactive at temperatures above 55°C, i.e. throughout the thermal cycling steps, and therefore does not destroy target amplicon formed during amplification.

Detection of PCR Products in a COBAS® TaqMan® Test

The COBAS® TaqMan® HBV Test utilizes real-time[27,28] PCR technology. The use of dual-labeled fluorescent probes provides for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HBV and HBV QS-specific oligonucleotides labeled with a reporter dye and a quencher dye. In the COBAS® TaqMan® HBV Test, the HBV and HBV Quantitation Standard probes are labeled with different fluorescent reporter dyes. When the dual fluorescent dye-labeled probes are intact, the reporter fluorescence is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' → 3' nuclease activity of the thermostable Z05 DNA polymerase. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HBV DNA and HBV QS DNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HBV DNA and HBV QS DNA. The intensity of the signals is related to the amount of starting material at the beginning of the PCR.

Fundamentals of COBAS® TaqMan® HBV Test Quantitation

The COBAS® TaqMan® HBV Test accurately provides quantitative results over a very wide dynamic range since the monitoring of amplicon is performed during the exponential phase of amplification. The higher the HBV titer of a specimen, the earlier the fluorescence of the reporter dye of the HBV probe rises above the baseline fluorescence level (*see* Figure 1). Since the amount of HBV QS DNA is constant between all specimens, the fluorescence of the reporter dye of the HBV QS probe should appear at the same cycle for all specimens (*see* Figure 2). In cases where the QS amplification and detection is affected by inhibition or poor specimen recovery, the appearance of fluorescence will be delayed, thereby enabling the calculated titer of HBV target DNA to be adjusted accordingly. The appearance of the specific fluorescent signal is reported as a critical threshold value (Ct). The Ct is defined as the fractional cycle number where reporter dye fluorescence exceeds a predetermined threshold (the Assigned Fluorescence Level), and starts the beginning of an exponential growth phase of this signal (*see* Figure 3). A higher Ct value indicates a lower titer of initial HBV target DNA. A 2-fold increase in titer correlates with a decrease of 1 Ct for target HBV DNA, while a 10-fold increase in titer correlates with a decrease of 3.3 Ct. Figure 1 depicts the target growth curves for a dilution series of virus spanning over a 5-$\log_{10}$ range. As the concentration of the virus increases, the growth curves shift to earlier cycles. Therefore the leftmost growth curve corresponds to the highest viral titer level whereas the rightmost growth curve corresponds to the lowest viral titer level.

Figure 1
Target Growth Curves for a Dilution Series of Virus Spanning over a 5-log_{10} Range

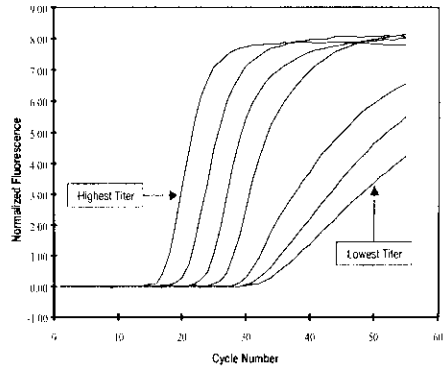


Figure 2 depicts the Quantitation Standard growth curves for specimens from a viral dilution series that spans a 5-log_{10} range. The amount of Quantitation Standard added to each specimen is constant for each reaction. The Ct value of the Quantitation Standard is similar regardless of the viral titer.

Figure 2
Quantitation Standard Growth Curves for a Dilution Series of Virus Spanning over a 5-log_{10} Range

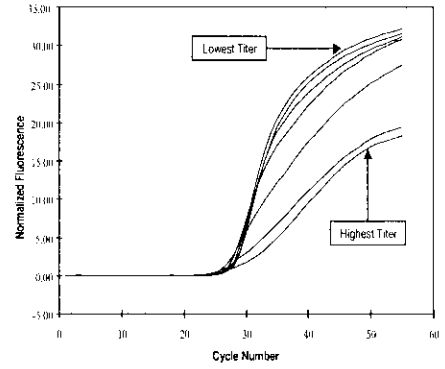
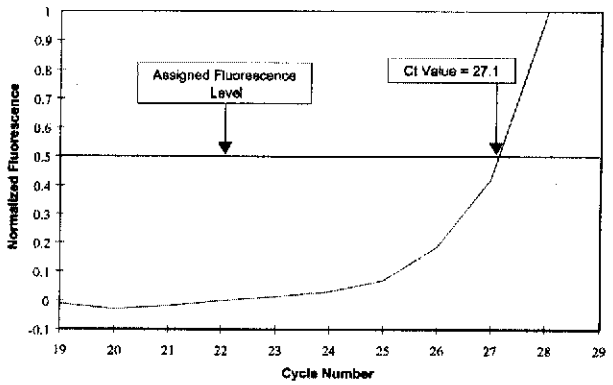


Figure 3 provides an example of how the fluorescence values at every cycle are normalized for each growth curve. The fractional cycle number (Ct) is calculated where the fluorescence signal crosses the Assigned Fluorescence Level.

Figure 3
Fluorescence Values at Every Cycle are Normalized for Each Growth Curve



HBV DNA Quantitation

The COBAS® TaqMan® HBV Test quantitates HBV viral DNA by utilizing a second target sequence (HBV Quantitation Standard) that is added to each test specimen at a known concentration. The HBV Quantitation Standard is a non-infectious, linearized, double stranded plasmid DNA construct, containing fragments of HBV sequences with primer binding regions identical to those of the HBV target sequence. The HBV Quantitation Standard also generates an amplification product of the same length and base composition as the HBV target DNA. The detection probe binding region of the HBV Quantitation Standard has been modified to differentiate HBV Quantitation Standard amplicon from HBV target amplicon.

During the annealing phase of the PCR on the COBAS® TaqMan® 48 Analyzer, the specimens are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the HBV DNA and HBV Quantitation Standard DNA data represent sets that are valid, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the HBV DNA and the HBV Quantitation Standard DNA. The lot-specific calibration constants provided with the COBAS® TaqMan® HBV Test are used to calculate the titer value for the specimens and controls based upon the HBV DNA and HBV Quantitation Standard DNA Ct values. The COBAS® TaqMan® HBV Test is standardized against the WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Technology (NAT) Assays Testing NIBSC 97/746[29] and titer results are reported in International Units (IU/mL).

REAGENTS

High Pure System Viral Nucleic Acid Kit 48 Tests
(P/N: 03502295 001)

LYS **2 x 25 mL**
(Lysis/Binding Buffer)
Tris
52% Guanidine-HCl
< 1% Urea
20% Triton X-100
Xn  52% (w/w) Guanidine-HCl
Harmful

CAR **2 x 2 mg**
(RNA, lyophilized)

PK **2 x 100 mg**
(Proteinase K, lyophilized)
99% Proteinase K, lyophilized
Xn  99% (w/w) Proteinase K, lyophilized
Harmful

IRB **1 x 33 mL**
(Inhibitor Removal Buffer)
Tris
65% Guanidine-HCl
(add 20 mL Ethanol)
Xn  65% (w/v) Guanidine-HCl
Harmful

WASH **1 x 20 mL**
(Wash Buffer)
Tris
NaCl
(add 80 mL Ethanol)

ELB **1 x 30 mL**
(Elution Buffer)

RS **4 x each**
(High Pure System Viral Nucleic Acid Rack Set)
Lysis Rack
Filter Tube Rack with affixed Waste Rack
Elution Rack
Cover Rack
Grippers

WR **8 x each**
(High Pure System Viral Nucleic Acid Waste Rack)

Specimen Preparation and Control Reagents

HBV QS **2 x 1.0 mL**
(HBV Quantitation Standard)
- Tris-HCl buffer
- EDTA
- < 0.001% linearized, double stranded plasmid DNA containing an insert. The DNA insert contains HBV primer binding sequences and a unique probe binding region.
- Amaranth dye
- < 0.005% Poly rA RNA (synthetic)
- 0.05% Sodium azide

HBV H(+)C **2 x 1.0 mL**
[HBV High (+) Control]
- < 0.001% linearized, double stranded plasmid DNA containing HBV sequences. Lot specific concentration range can be found on the COBAS® TaqMan® HBV Test Controls Value Card.
- Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
- 0.1% ProClin® 300 preservative

HBV L(+)C **2 x 1.0 mL**
[HBV Low (+) Control]
- < 0.001% linearized, double stranded plasmid DNA containing HBV sequences. Lot specific concentration range (close to the assay LoD) can be found on the COBAS® TaqMan® HBV Test Controls Value Card.
- Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
- 0.1% ProClin® 300 preservative

CTM (–) C **4 x 1.0 mL**
[COBAS® TaqMan® Negative Control (Human Plasma)]
- Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
- 0.1% ProClin® 300 preservative

Amplification and Detection Reagents

HBV MMX	**2 x 24 Tests**
(COBAS® TaqMan® HBV Master Mix)	2 x 1.4 mL
Tricine buffer	
Potassium hydroxide	
Potassium acetate	
Glycerol	
< 0.001% dATP, dCTP, dGTP, dUTP	
< 0.001% Upstream and downstream primers to the Pre-Core/Core region of HBV	
< 0.001% Fluorescent-labeled oligonucleotide probes specific for HBV and the HBV Quantitation Standard	
< 0.001% Oligonucleotide aptamer	
< 0.05% Z05 DNA Polymerase (microbial)	
< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)	
0.09% Sodium azide	

CTM Mn^{2+}	**2 x 24 Tests**
(COBAS® TaqMan® Manganese Solution)	2 x 1.0 mL
< 1.2% Manganese acetate	
Glacial acetic acid	
0.09% Sodium azide	

WARNINGS AND PRECAUTIONS

A. **FOR *IN VITRO* DIAGNOSTIC USE.**

B. This test is for use with human plasma collected in the anticoagulant EDTA and human serum.

C. Do not pipette by mouth.

D. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.

E. ***Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent bottles.***

F. ***The use of sterile disposable pipettes and DNase-free pipette tips is recommended.***

G. Do not pool reagents from different lots or from different bottles of the same lot.

H. Do not mix reagents from different kits.

I. Dispose of unused reagents, waste and specimens in accordance with country, federal, state and local regulations.

J. Do not use a kit after its expiration date.

K. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.

L. Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[10] and in the CLSI Document M29-A3.[11] Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

Note: ***Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.***

M. **CAUTION: CTM (–) C, HBV L(+)C** and **HBV H(+)C** contain Human Plasma derived from human blood. The source material has been tested by US FDA allowed tests and found non-reactive for the

presence of Hepatitis B Surface Antigen (HBsAg), antibodies to HIV-1/2 and HCV, and HIV p24 Antigen. Testing of Negative Human Plasma by PCR methods showed no detectable HIV-1 RNA, HCV RNA or HBV DNA. No known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all human sourced material should be considered potentially infectious. **CTM (–) C**, **HBV L(+)C** and **HBV H(+)C** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[30] and in the CLSI Document M29-A3.[31] Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

N. **HBV MMX**, **HBV QS** and **CTM Mn^{2+}** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide-containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

O. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.

STORAGE AND HANDLING REQUIREMENTS

Specimen Preparation Reagents

A. Store the High Pure System Viral Nucleic Acid Reagents at 15-25°C upon arrival.

B. Store Elution Buffer (**ELB**) and Lysis/Binding Buffer (**LYS**) at 15-25°C. Once opened, store **ELB** and **LYS** at 15-25°C. Opened **ELB** and **LYS** must be used within 30 days or until the expiration date, whichever comes first.

C. After addition of Elution Buffer (**ELB**) to reconstitute the Carrier RNA and Proteinase K, store unused reconstituted Carrier RNA (**CAR**) and unused reconstituted Proteinase K (**PK**) at -15 to -25°C. Once reconstituted, Carrier RNA and Proteinase K must be used within 30 days or until the expiration date, whichever comes first.

D. After addition of ethanol, store Inhibitor Removal Buffer (**IRB**) and Wash Buffer (**WASH**) at 15-25°C. These Working Solutions are stable for 30 days or until the expiration date, whichever comes first.

E. The Lysis/Binding Working Solution [Lysis/Binding Buffer with Carrier RNA, Proteinase K and **HBV QS**] must be used immediately following preparation. Any excess must be discarded.

PCR Reagents (Amplification and Detection)

A. ***Do not freeze reagents or controls.***

B. Store **HBV MMX**, **CTM (–) C**, **HBV L(+)C**, **HBV H(+)C**, **HBV QS** and **CTM Mn^{2+}** at 2-8°C. Unopened, these reagents are stable until the expiration date indicated. Once opened to remove an aliquot for a batch size of 12 specimens, store remaining **HBV MMX**, **HBV L(+)C**, **HBV H(+)C**, **HBV QS** and **CTM Mn^{2+}** at 2-8°C. Once opened, **HBV MMX**, **HBV L(+)C**, **HBV H(+)C**, **HBV QS** and **CTM Mn^{2+}** are stable at 2-8°C for 30 days or until the expiration date, whichever comes first. Once opened, any unused portion of **CTM (–) C** must be discarded.

C. Working Master Mix (prepared by the addition of **CTM Mn^{2+}** to **HBV MMX**) must be stored at 2-8°C in the dark. The prepared specimens and controls must be added within 2 hours of preparation of the Working Master Mix.

D. Processed specimens and controls are stable for up to 3 hours at 20-30°C, up to 24 hours at 2-8°C or frozen at -20°C for up to 1 week.

E. Amplification must be started within 3 hours from the time that the processed specimens and controls are added to the Working Master Mix.

MATERIALS PROVIDED

Specimen Preparation Reagents

A. **High Pure System Viral Nucleic Acid Kit**
(P/N: 03502295 001)

LYS
(Lysis/Binding Buffer)

CAR
(Carrier RNA)

PK
(Proteinase K)

IRB
(Inhibitor Removal Buffer)

WASH
(Wash Buffer)

ELB
(Elution Buffer)

RS
(High Pure System Viral Nucleic Acid Rack Set)

WR
(High Pure System Viral Nucleic Acid Waste Rack)

Amplification and Detection Reagents

B. **COBAS® TaqMan® HBV Test** **HBV HPS**
(P/N: 03577163 190)

HBV QS
(HBV Quantitation Standard)

HBV H(+)C
[HBV High (+) Control]

HBV L(+)C
[HBV Low (+) Control]

CTM (–) C
[COBAS® TaqMan® Negative Control (Human Plasma)]

HBV MMX
(COBAS® TaqMan® HBV Master Mix)

CTM Mn^{2+}
(COBAS® TaqMan® Manganese Solution)

MATERIALS REQUIRED BUT NOT PROVIDED

Instrumentation and Software

- COBAS® TaqMan® 48 Analyzer
- AMPLILINK Software, Version 3.2 series and the AMPLILINK Software Version 3.2 Series Application Manual For use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, and COBAS® AMPLICOR® Analyzer
- Data station for the AMPLILINK software
- COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.2 Series Application Manual
- K-tube capper

Disposables

- K-tube Box of 12 x 96

Centrifuge Requirements

- Sigma 4-15°C Benchtop centrifuge or equivalent micro-titer plate centrifuge capable of delivering 4600 x g centrifugal force
- Sigma centrifuge swing out rotor P/N: 11118 (includes 2 buckets P/N: 13218 and 2 plate holders P/N: 17978) or equivalent

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

- Isopropanol (> 99%) - meets ACS specifications or better
- Ethanol (96 - 100%) - meets ACS specifications or better
- Adjustable Pipettors*: (capacity 250 µL and 1000 µL) with aerosol barrier or positive displacement DNase-free tips
- Pipette Aid: Drummond (P/N: 4-000-100) or equivalent
- Water Bath set at 50°C ± 2°C
- Dry Heat Block set at 70°C
- Sterile disposable, serological pipettes: 5, 10 and 25 mL
- Sterile polypropylene conical tubes: 15 mL and 50 mL: Corning (P/N: 430052 and P/N: 430290) or equivalent
- Sterile 2.0 mL microfuge tubes: Sarstedt (P/N: 72.693.005) or equivalent
- Vortex mixer
- Tube racks
- Disposable gloves, powderless
- Calibrated Thermometers for Water Bath and Dry Heat Block

* *Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement DNase-free tips must be used where specified to prevent specimen and amplicon cross-contamination.*

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Note: Handle all specimens and controls as if they are capable of transmitting infectious agents.

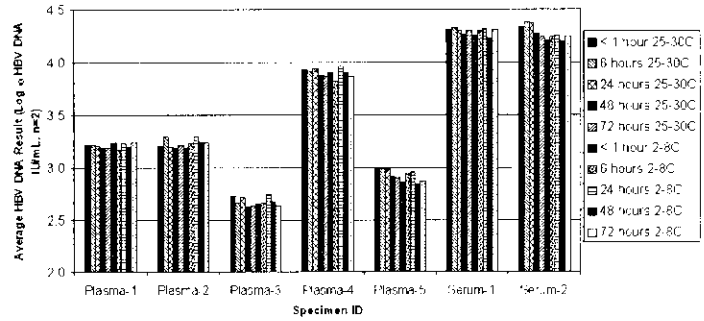
A. Specimen Collection

The COBAS® TaqMan® HBV Test is for use with serum or EDTA plasma specimens only. Blood should be collected in BD SST Serum Separator Tubes or in tubes using EDTA (lavender top) as the anticoagulant.

Store whole blood at 2-25°C for no longer than 1 day. The use of EDTA plasma or serum will yield test results that are similar. *See* Figure 11 in the Non-Clinical Performance Evaluation section, entitled "Regression Analysis of Matched Serum – EDTA Plasma Samples (n=50)", which demonstrates the effect of matrix type on HBV DNA results from patient specimens.

Separate serum or plasma from whole blood within 1 day of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature. Transfer serum or plasma to a sterile polypropylene tube. Figure 4 illustrates the data from these specimen collection studies. The largest observed differences between the EDTA plasma conditions was not more than ± 0.12 $\log_{10}$ and the largest observed differences between the serum conditions was not more than ± 0.16 $\log_{10}$.

Figure 4
HBV Stability in Whole Blood With EDTA Anticoagulant or in Serum Separator Tubes Before Separation into Plasma or Serum



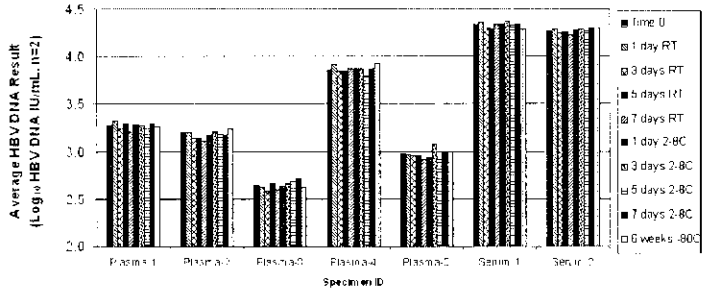
B. Specimen Transport

Transportation of whole blood, serum or plasma must comply with country; federal, state and local regulations for the transport of etiologic agents.[32] Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma or serum may be transported at 2-8°C or frozen at -20°C to -80°C.

C. Specimen Storage

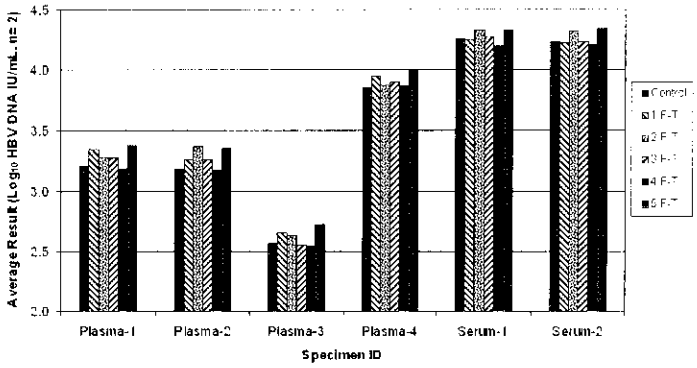
Serum or plasma specimens may be stored at room temperature for up to 3 days, at 2-8°C for up to 7 days or frozen at -20°C to -80°C for up to six weeks. The largest observed differences between the EDTA plasma conditions was not more than ± 0.11 $\log_{10}$ and the largest observed differences between the serum conditions was not more than ± 0.05 $\log_{10}$ across the tested conditions. It is recommended that specimens be stored in 800-900 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as Sarstedt P/N: 72.694.006). Figure 5 shows the data from these specimen storage studies.

Figure 5
HBV Stability in EDTA-Plasma or Serum



Serum and plasma specimens may be frozen and thawed up to five times without a loss of HBV DNA. The largest observed differences between the EDTA plasma conditions was not more than ± 0.19 $\log_{10}$ and the largest observed differences between the serum conditions was not more than ± 0.10 $\log_{10}$. Figure 6 illustrates the data from these freeze-thaw studies.

Figure 6
HBV Results After Up to Five Freeze-Thaw (F-T) Cycles



INSTRUCTIONS FOR USE

Note: For detailed operating instructions, printing results and interpreting flags, comments and error messages, refer to the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.2 Series Application Manual and the AMPLILINK Software Version 3.2 Series Application Manual For use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, and COBAS® AMPLICOR® Analyzer.

Note: All amplification and detection reagents must be at 15 – 30°C before use; remove from 2-8°C storage at least 30 minutes before use.

Note: Serum and plasma specimens must be equilibrated for 15 - 30 minutes at 15 – 30°C before use.

Note: Use pipettors with aerosol barrier or positive displacement tips where specified. Use extreme care to avoid contamination.

Run Size

Each kit contains reagents sufficient for four 12-test runs, which may be performed separately or simultaneously. One replicate each of the **CTM (–) C**, **HBV L(+)C** and the **HBV H(+)C** must be included in each test run of up to 24 specimens and controls (see "*Quality Control*" section). The Amplification and Detection Reagents are packaged in 24-test, dual-use bottles. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12.

Specimen and Positive and Negative Control Preparation

Note: If using frozen serum or plasma specimens, place the specimens at room temperature until completely thawed and vortex for 5 - 10 seconds before use.

Note: Allow reagents to reach 15 – 30°C before proceeding. Preheat Heating Block(s) to a temperature of 70°C and water bath to a temperature of 50°C before starting the purification reactions.

A. Reagent Preparation

Note: Prepare the Lysis/Binding Working Solution only after all specimens and controls have been equilibrated at 15 – 30°C for 15 - 30 minutes.

1. Prepare the Inhibitor Removal Buffer by pipetting 20 mL of 96 - 100% ethanol to Inhibitor Removal Buffer (**IRB**). Mix by inverting 5 - 10 times. This is enough diluted Inhibitor Removal Buffer for 48 tests.

2. Prepare the Wash Buffer by pipetting 80 mL of 96 - 100% ethanol to the Wash Buffer (**WASII**). Mix by inverting 5 - 10 times. This is enough diluted Wash Buffer for 48 tests.

3. Preheat the Elution Buffer (**ELB**) at 70°C in a 2.0 mL screw-cap microfuge tube. Multiple tubes can be used. The elution volume per specimen is 75 µL. Pre-warm the volume listed in the table below according to the number of tests.

	Number of Replicates	
Reagent	**12**	**24**
Elution Buffer (mL)	2.0	4.0

4. Pipette the volume of isopropanol listed in the table below according to number of tests, into a clean sterile tube.

	Number of Replicates	
Reagent	**12**	**24**
Isopropanol (mL)	5.0	10.0

5. Pipette 0.5 mL of the Elution Buffer (**ELB**) into the Carrier RNA (**CAR**). Replace the stopper; invert the vial, then vortex until all of the Carrier RNA is dissolved. The reconstituted Carrier RNA is enough for 24 tests. Unused volume of reconstituted Carrier RNA may be stored at -15 to -25°C for up to 30 days or until the expiration date, whichever comes first.

6. Pipette 5.0 mL of the Elution Buffer (**ELB**) into the Proteinase K (**PK**). Replace the stopper; invert the vial, then vortex until all of the Proteinase K is dissolved. The reconstituted Proteinase K is enough for 24 tests. Unused volume of reconstituted Proteinase K may be stored at -15 to -25°C for up to 30 days or until the expiration date, whichever comes first.

7. Prepare the Lysis/Binding Working Solution as follows by pipetting the volumes listed in the following table according to the number of specimens and controls to be processed:

	Number of Replicates	
Reagent	**12**	**24**
Lysis/Binding Buffer (mL)	7.0	14.0
Carrier RNA (µL)	140	280
HBV QS (µL)	39	78
Proteinase K (mL)	1.4	2.8

Note: Volume of HBV QS is specific to the COBAS® TaqMan® HBV Test.

Note: If frozen reconstituted Carrier RNA or Proteinase K are to be used, thaw at room temperature, and invert several times prior to use.

- Add the indicated volume of Lysis/Binding Buffer to a clean sterile 50 mL tube.
- Add the indicated volume of reconstituted Carrier RNA to the tube containing the Lysis/Binding Buffer.
- Vortex the **HBV QS** for 3-5 seconds and add indicated volume of **HBV QS** to the tube containing the Lysis/Binding Buffer and the reconstituted Carrier RNA.
- Cap the tube and mix by inverting 10-15 times. **Do NOT vortex** - vortexing will create bubbles in the solution.
- Add the indicated volume of reconstituted Proteinase K to the tube containing the Lysis/Binding Buffer.

- Cap the tube and mix by inverting 10-15 times. **Do NOT vortex** — vortexing will create bubbles in the solution. Start dispensing the Lysis/Binding Working Solution immediately after the addition and mixing of the Proteinase K with the Lysis/Binding Buffer.

- Unused Lysis/Binding Buffer (**LYS**) may be stored at 15-25°C for up to 30 days or until the expiration date, whichever comes first. Unused Lysis/Binding Working Solution must be discarded.

B. Specimen and Control Preparation

*Note: **Adjustable pipettors with aerosol-resistant tips are recommended for this procedure.***

*Note: **A repeat pipette with properly sized sterile combi-tips can be used at Steps 14, 16, 19 and 22. However, extra care should be taken to avoid splashing of reagents and cross-contamination.***

1. Pipette 625 µL of Lysis/Binding Working Solution into each well of the Lysis Rack (I, transparent). Push the lids down into the covering position.

2. Opening one well at a time, pipette 500 µL of specimen or control into the correct well. After addition of each specimen or control, push the lid down until the snap mechanism engages to tightly close the well.

3. After all specimens and controls have been added, mix by vortexing the filled Lysis Rack for approximately 10 seconds. Visually confirm all wells are being vortexed and well mixed.

4. Incubate the Lysis Rack in a preheated 50°C ± 2.0 °C water bath for 10 minutes. Float the Lysis Rack in a water bath that is filled with approximately 5-7 cm of water. Dry Lysis Rack after removing from water bath.

5. Centrifuge the Lysis Rack for 10-20 seconds at a setting of 4600 x g in the micro-titer plate centrifuge. The centrifuge may not reach set speed.

6. Opening one well at a time, pipette 250 µL of isopropanol into each well. After each addition, push the lid down until the snap mechanism engages to tightly close the well.

*Note: **Volume of isopropanol is specific to each COBAS® TaqMan® Test.***

7. Mix specimens by inverting the rack three times, then vortexing the rack for approximately 10 seconds. Visually confirm all wells are being vortexed and well mixed.

8. Centrifuge the Lysis Rack for 10-20 seconds at a setting of 4600 x g in the micro-titer plate centrifuge. The centrifuge may not reach set speed.

9. Opening one well at a time, transfer 750 µL of specimen or control mixture to the corresponding wells of the Filter Tube Rack (II, yellow) with affixed Waste Rack (white). After the addition of each specimen or control mixture, push the lid down until the snap mechanism engages to tightly close the well.

10. After all specimens or controls have been added, centrifuge the Filter Tube Rack assembly for 2 minutes at 4600 x g in the micro-titer plate centrifuge.

11. Opening one well at a time, transfer the remaining specimen or control mixture to the corresponding wells of the Filter Tube Rack. After the addition of each specimen or control mixture, tightly close the lid of the well. Discard the Lysis Rack.

12. Centrifuge the Filter Tube Rack assembly for 2 minutes at 4600 x g in the micro-titer plate centrifuge.

13. Remove the Filter Tube Rack from the Waste Rack by pressing both snap links on the upper side of the Filter Tube Rack. Discard the Waste Rack. Replace with a new Waste Rack and snap the Filter Tube Rack onto the Waste Rack.

14. Open one well at a time and pipette 400 µL of Inhibitor Removal Buffer (**IRB**) down the side of each well. **Do not touch the sides of the well.** After the addition of Inhibitor Removal Buffer to all wells, tightly close the lids.

15. Centrifuge the Filter Tube Rack assembly for 2 minutes at 4600 x g in the micro-titer plate centrifuge.

16. Open one well at a time and pipette 700 µL of Wash Buffer (**WASII**) down the side of each well. **Do not touch the sides of the well.** After the addition of Wash Buffer to all wells, tightly close the lids.

17. Centrifuge the Filter Tube Rack assembly for 2 minutes at 4600 x g in the micro-titer plate centrifuge.

18. Remove the Filter Tube Rack from the Waste Rack by pressing both snap links on the upper side of the Filter Tube Rack. Discard the Waste Rack. Replace with a new Waste Rack and snap the Filter Tube Rack onto the Waste Rack.

19. Open one well at a time and pipette 700 µL of Wash Buffer down the side of each well. **Do not touch the sides of the well.** After the addition of Wash Buffer to all wells, tightly close the lids.

20. Centrifuge the Filter Tube Rack assembly for 3 minutes at 4600 x g in the micro-titer plate centrifuge.

21. Remove the Filter Tube Rack from the Waste Rack by pressing both snap links on the upper side of the Filter Tube Rack. Place the Filter Tube Rack onto the Elution Rack (IIIA, blue) and snap the Filter Tube Rack onto the Elution Rack. Discard the Waste Rack appropriately.

22. Open one well at a time and pipette 75 µL of the pre-warmed Elution Buffer **(ELB) onto the center of each filter without touching the filter**.

Note: ***Do not add Elution Buffer by dispensing down the side of the well.***

23. After the addition of Elution Buffer to all wells, tightly close the lids. Incubate the Elution Rack at room temperature for a minimum of 3 minutes after adding Elution Buffer to the last well.

24. Centrifuge the Filter Tube Rack assembly for 3 minutes at 4600 x g in the micro-titer plate centrifuge.

25. Remove the Filter Tube Rack from the Elution Rack by pressing both snap links on the upper side of the Filter Tube Rack. Discard the Filter Tube Rack appropriately.

26. Place the Cover Rack (IIIB, blue) onto the Elution Rack (IIIA, blue). Press down firmly and snap links onto the Elution Rack. Close all lids.

27. The processed specimens and controls are used directly for PCR. Use 50 µL of the processed specimens and controls for amplification. Add the processed specimens and controls to the Working Master Mix within 3 hours of completing specimen and control preparation. If processed specimens and controls cannot be used within 3 hours of their preparation, the processed specimens and controls can be stored at 2-8°C for up to 24 hours in the covered Elution Rack or frozen at -20°C for up to 1 week in sterile 2.0 mL polypropylene screw-cap tubes (such as Sarstedt P/N: 72.694.006).

Amplification and Detection

Note: ***K-carriers and K-carrier holder should be wiped with a lint free cloth moistened with a 70% isopropanol solution.***

C. Reagent Preparation

Note: ***COBAS® TaqMan® HBV Master Mix (HBV MMX), "Working Master Mix" (Working MMX) and Working Master Mix plus processed specimens and controls are light sensitive. Protect these reagents from light.***

Note: ***COBAS® TaqMan® HBV Master Mix (HBV MMX) and COBAS® TaqMan® Manganese Solution (CTM Mn^{2+}) must be equilibrated at 15 – 30°C for at least 30 minutes prior to preparation of Working Master Mix.***

Note: ***Prepare Working MMX after completion of Specimen and Control Preparation.***

Note: ***The Working MMX must be used within 2 hours of preparation***

Note: ***Once processed specimens and controls are added to Working Master Mix, amplification must be started within 3 hours.***

1. Equilibrate one vial of **HBV MMX** and one vial **CTM Mn^{2+}** at 15 – 30°C for at least 30 minutes.

2. Place a K carrier in a K-carrier holder.

3. Place new K-tubes in the K-carrier without touching the sides of the K-tubes.

Note: ***If fewer than 24 tubes are to be run, positions 1, 2, 5, 20, 23 and 24 must be occupied to balance the K-carrier in the Thermal Cycler.***

4. Uncap the K-tubes using the K-tube Capper. Place caps in the K-tube Park Position Holder.

5. Prepare the Working MMX as follows:

   For 24 tests, add 191 µL of **CTM Mn^{2+}** to one vial of **HBV MMX**. Cap the bottle and mix well by inverting 10 times. Do not vortex the Working MMX. Protect the Working MMX from light and use within 2 hours.

   For 12 tests, remove 660 µL of **HBV MMX** and place in a 2 mL tube. Add 90 µL of **CTM Mn^{2+}** to the 2 mL tube containing **HBV MMX**, cap the tube and mix well by inverting 10 times. Protect the Working MMX from light and use within 2 hours. Store the remainder of the unused **HBV MMX** and **CTM Mn^{2+}** in the original vials at 2-8°C. Once opened, **HBV MMX** and **CTM Mn^{2+}** are stable at 2-8°C for 30 days or until the expiration date, whichever comes first.

Note: ***Volume of CTM Mn^{2+} is specific to the COBAS® TaqMan® HBV Test.***

6. Pipette 50 µL of Working MMX into each K-tube.

Note: ***If processed specimens and controls were stored frozen prior to amplification, thaw at room temperature before proceeding to Step 7.***

7. Add 50 µL of each processed specimen and control to the proper K-tube containing Working MMX using a micropipettor with an aerosol barrier or positive displacement tip. Gently mix each specimen or control up and down three times with the micropipettor without generating bubbles.

8. Repeat Step 7 for each processed specimen and processed control until all have been transferred to K-tubes. Use a new tip for each specimen and control. Visually inspect for bubbles and remove as necessary. Cap the K-tubes using a K-tube Capper. Visually verify correct volumes have been added.

9. Amplification must be started within 3 hours of the time that the processed specimens and controls are added to the K-tubes containing the Working MMX.

D. COBAS® TaqMan® 48 Analyzer Loading and Operation

1. Turn on the workstation computer and log onto Windows XP using the correct User ID and password.

2. Turn on the COBAS® TaqMan® 48 Analyzer. Verify that the instrument initializes and is ready for use. If K-carriers from previous run(s) are still located in either of the Thermal Cyclers, remove them using the K-carrier transporter.

3. Open AMPLILINK software on the computer. Log on using the correct User ID and password.

4. To create K-carrier orders for the specimens to be analyzed, click on the **Orders** icon. Select the **Sample** tab, and then click on the **New** button and enter the order number for each specimen using the keypad or barcode scanner. Select the Test Definition for the COBAS® TaqMan® HBV Test. Repeat for each specimen. Click the **Save** button.

Note: ***If fewer than 24 tubes are to be run, positions 1, 2, 5, 20, 23 and 24 must be occupied to balance the K-carrier in the Thermal Cycler.***

5. Enter Quality Control information by selecting the **Quality Control** tab in the **Orders** window. Click the **New** button and enter the information from the **COBAS® TaqMan® HBV Test Controls Value Card** supplied with the kit using the keypad or barcode scanner. Enter the COBAS® TaqMan® HBV Test lot number, expiration date, Low (+) and High (+) Control ranges as well as lot-specific calibration coefficients in the designated spaces. Click "**OK**."

6. Assign a K-carrier number for the run by clicking on the **K-Carrier** tab in the **Orders** window. In the **K-Carrier** window, click **New**. In the cell to the right of "**K-Carrier ID,**" enter the K-carrier number from the barcode on the K-carrier using the keypad or barcode scanner. Note that the results from a previous run with the same K-Carrier ID must be accepted. Select the Test Definition for the COBAS® TaqMan® HBV Test from the test panel at the lower portion of the window.

7. In the **Worklist**, select the first row of the Type (T) column. Highlight this field to access the pull down menu and then select the required control type. Next, double click the sample ID field for the same row. The **LookUp Control** Window will be displayed with all available controls. When the control is selected, the corresponding calibration and control values will be displayed in the lower right Information panel. Repeat this process for all required controls.

8. To enter specimens to the **Worklist**, double-click on the first position (row) for specimen entry. This will display the **Lookup Sample** window containing the assigned specimen orders. Use the **Shift + Arrow** keys to highlight more than one order number. Verify that all orders have been assigned the Test Definition for the COBAS® TaqMan® HBV Test.

9. Click **Save** to save the K-carrier order assignment.

E. Amplification and Detection

10. Select the **Systems** Icon in the System Tab; click **Open** to open the Thermal Cycler. When the Thermal Cycler Cover has completely opened and "**Ready to Load**" is seen in the **Systems** window, lift and hold the Thermal Cycler lid open. Using the K-carrier Transporter, transfer the loaded K-carrier containing the capped K-tubes with Working Master Mix and specimens and controls into the Thermal Cycler. Close the Thermal Cycler lid.

11. Click **Start** on the **Systems** window below the TC icon to close the Thermal Cycler Cover and start the run.

12. Amplification and detection are automatically performed by the COBAS® TaqMan® 48 Analyzer.

RESULTS

Result Calculation

The COBAS® TaqMan® 48 Analyzer automatically determines the HBV DNA titer for the specimen or control. The **HBV DNA titer is expressed in International Units (IU)/mL**. The conversion factor between HBV copies/mL and HBV International Units/mL is 5.82 copies/IU using the WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Technology (NAT) Assays Testing NIBSC 97/746.[20]

The COBAS® TaqMan® 48 Analyzer:

- Determines the Cycle Threshold value (Ct) for the HBV DNA and the HBV Quantitation Standard DNA.
- Determines the HBV DNA titer based upon the Ct values for the HBV DNA and HBV Quantitation Standard DNA and the lot-specific calibration coefficients.
- Determines that the calculated IU/mL titers for **HBV L(+)C** and **HBV H(+)C** fall within the assigned ranges stated on the COBAS® TaqMan® HBV Test Controls Value Card supplied with the kit.

Run Validation

Check AMPLILINK results window or printout for flags and comments to ensure that the run is valid.

The run is valid if no flags appear for the COBAS® TaqMan® HBV Controls. The following results are obtained for a valid run:

Control	Result	Interpretation
Negative Control	Target Not Detected	Control within range
Low Positive Control	A numeric titer X.XXE+XX IU/mL	Control within range
High Positive Control	A numeric titer X.XXE+XX IU/mL	Control within range

The run is <u>not</u> valid if any of the following flags appear for the COBAS® TaqMan® HBV Controls:

Negative Control

Flag	Result	Interpretation
_N_NC_INVALID	Invalid	An invalid result

HBV Low Positive Control

Flag	Result	Interpretation
_L_LPCINVALID	< 2.90E+01 IU/mL	An invalid result where the Control was below the assay range
	A numeric titer, X.XXE+XX IU/mL	An invalid result where the Control was out of the assigned control range
	> 1.10E+08 IU/mL	An invalid result where the Control was above the assay range
	Invalid	An invalid result

HBV High Positive Control

Flag	Result	Interpretation
_H_HPCINVALID	< 2.90E+01 IU/mL	An invalid result where the Control was below the assay range
	A numeric titer, X.XXE+XX IU/mL	An invalid result where the Control was out of the assigned control range
	> 1.10E+08 IU/mL	An invalid result where the Control was above the assay range
	Invalid	An invalid result

If the run is invalid, repeat the entire run including specimen and control preparation, amplification and detection.

Interpretation of Results:

For a valid run, check each individual specimen for flags or comments on the result printout. Interpret the results as follows:

- A valid run may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

Titer Result	Interpretation
Target Not Detected	No Ct value for HBV obtained. Report results as "HBV DNA not detected".
< 2.90E+01 IU/mL	Below 2.90E+01 IU/mL (lower limit of quantitation, LLoQ). HBV DNA is not quantifiable.
≥ 2.90E+01 IU/mL and ≤ 1.10E+08 IU/mL	Calculated results greater than or equal to 29 IU/mL and less than or equal to 1.1E+08[1] IU/mL are within the Linear Range of the Assay.
> 1.10E+08 IU/mL	IU/mL are above the Linear Range of the assay. Report results as "greater than 1.10E+08 HBV DNA IU/mL". If quantitative results are desired, the original specimen should be diluted 1:100 with HBV-negative human plasma or serum depending upon the matrix of the original specimen (plasma samples must be diluted in plasma and serum samples must be diluted in serum), and the test repeated. Multiply the reported result by the dilution factor.

[1] *1.1E+08 IU/mL = $1.1x10^8$ IU/mL*

*Note: **In some rare instances, specimens with very high titers can produce an Invalid result with a flag "QS_INVALID." The result for these samples is not valid and must be repeated. If the sample continues to produce an invalid result the user can dilute the sample 1:100 in HBV negative plasma (for plasma samples) or HBV negative serum (for serum samples) in order to try to obtain a valid result. Multiply the reported result by the dilution factor.***

QUALITY CONTROL

One replicate each of the COBAS® TaqMan® Negative Control, the COBAS® TaqMan® HBV Low (+) Control and the COBAS® TaqMan® HBV High (+) Control must be included in each test run. The batch is valid if no flags appear for any of the controls [**HBV L(+)C, HBV H(+)C** and **CTM (-) C**].

Based on the results of a carry-over contamination study with alternating high positive HBV samples and HBV negative samples, there are no requirements regarding the position of the controls in the K-carrier. Check the run printout for flags and comments to ensure that the run is valid.

Negative Control

CTM (–) C must yield a "**Target Not Detected**" result, i.e. no Ct value for HBV DNA was obtained, but a valid Ct value was obtained for the HBV Quantitation Standard DNA. If **CTM (–) C** does not meet this criteria, the entire run is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If **CTM (–) C** is consistently not valid, contact your local Roche office for technical assistance.

Positive Controls

The assigned range for **HBV L(+)C** and **HBV H(+)C** is specific for each lot of control and is provided on the *COBAS® TaqMan® HBV Test Controls Value Card* supplied in the kit. These ranges are entered into the Data Station for the AMPLILINK software using the COBAS® TaqMan® 48 Analyzer barcode scanner or keypad.

The HBV DNA IU/mL for both the **HBV L(+)C** and **HBV H(+)C** must fall within the range indicated on the *COBAS® TaqMan® HBV Test Controls Value Card* supplied in the kit. If one or both of the positive controls does not meet these criteria, then the entire run is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the calculated HBV DNA titer of one or both of the positive controls is consistently outside the assigned range, contact your local Roche office for technical assistance.

PROCEDURAL PRECAUTIONS

1. As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored for purity. Discard any reagents that may be suspect.

PROCEDURAL LIMITATIONS

1. This test has been validated for use with human serum or human plasma collected in EDTA anticoagulant. Testing of other specimen types may result in incorrect results, false negative or false positive results.

2. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.

3. The presence of AmpErase enzyme in the COBAS® TaqMan® HBV Master Mix reduces the risk of amplicon contamination. However, contamination from HBV positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Package Insert.

4. Though rare, mutations within the highly conserved region of the viral genome covered by the COBAS® TaqMan® HBV Test For Use With The High Pure System primers and/or probe may result in the under quantitation of or failure to detect the virus.

5. Use of this product should be limited to personnel trained in the techniques of PCR.

6. This product can only be used with the COBAS® TaqMan® 48 Analyzer.

7. This test may cross-react with HPV Type 18 and CMV.

8. The linearity of the test was established only with HBV Genotype A

9. Precision was established with only Genotype A and Genotype C.

10. Detection of the HBV DNA target is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors (i.e., age, presence of symptoms and/or stage of infection).

11. If another assay was initially used for quantitation of treatment effect on the patient, it is recommended that prior to switching to the COBAS® TaqMan® HBV assay users perform method correlation studies in their laboratory to quantify technology differences.

NON-CLINICAL PERFORMANCE EVALUATION

Study Description

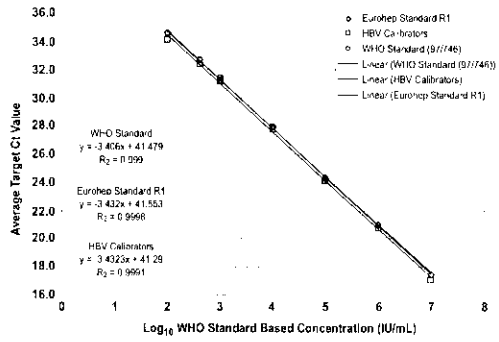
The analytical performance characteristics of the COBAS® TaqMan® HBV Test For Use With The High Pure System were determined in a series of studies described below. The Limit of Detection of the COBAS® TaqMan® HBV Test For Use With The High Pure System was determined for HBV DNA using several dilutions of the WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Technology (NAT) Assays Testing (NIBSC 97/746; genotype A)[19] and clinical specimens with genotypes A through G.

The linearity and precision of the COBAS® TaqMan® HBV Test For Use With The High Pure System was determined by analysis of serial dilutions of clinical HBV specimens in HBV-negative human EDTA-plasma and human serum. The clinical specimens' concentration (stock concentrations) determinations are traceable to the WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Technology (NAT) Assays Testing (NIBSC 97/746).

Traceability to the WHO Standard

Several standards and controls have been used during development of this test to provide traceability to the WHO Standard. This includes the Eurohep R1 standard[42], the WHO Standard[20], and RMS HBV Calibrators. The Eurohep R1 standard and the COBAS® TaqMan® HBV Test calibrators behave similarly relative to the WHO Standard, as shown in Figure 7.

Figure 7
Traceability of the COBAS® TaqMan® HBV Test For Use With
The High Pure System HBV Calibrators (2-7 on log_{10}) to the WHO Standard (range 2-5 on log_{10})
and Eurohep Standard R1 (range 2-7 on log_{10})



Linear Range

The linear range study was evaluated in accordance with the methods defined in the CLSI (formerly NCCLS) Guideline EP6-A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline."[45] As shown in Figure 8 and Figure 9, the COBAS™ TaqMan® HBV Test For Use With The High Pure System was found to give a linear response from 2.9E1 (log_{10} = 1.46) HBV DNA IU/mL to 1.10E8 (log_{10} = 8.02) HBV DNA IU/mL in both EDTA plasma and serum with deviation from linearity not more than 0.20 log_{10}, in both matrices. The study was performed using two lots of COBAS® TaqMan® HBV Test For Use With The High Pure System reagents and serial dilutions of an HBV positive genotype A specimen that was assigned relative to the WHO Standard. Twenty-seven replicates were tested per level in EDTA plasma and in serum.

Figure 8
Linearity of the COBAS® TaqMan® HBV Test
For Use With The High Pure System in EDTA Plasma for Genotype A

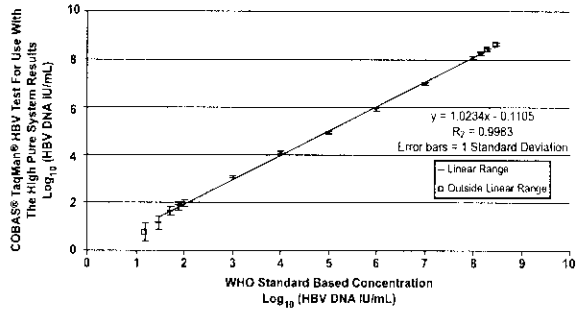
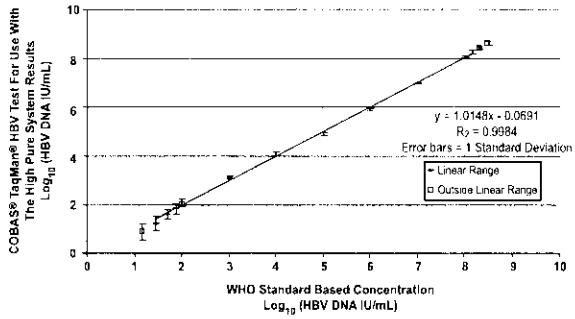


Figure 9
Linearity of the COBAS® TaqMan® HBV Test
For Use With The High Pure System in Serum for Genotype A



Linearity for genotypes other than genotype A was not evaluated.

The analytical measurement range of analyte values that can be directly measured on a specimen without any dilution using the COBAS® TaqMan® HBV Test is 29 to 1.1E+08 IU/mL.

The clinically reportable range of analyte values that can be measured on a specimen with a maximum dilution of one to one-hundred using the COBAS® TaqMan® HBV Test is 29 to 1.1E+10 IU/mL.

Inclusivity

Genotype Titer Quantitation

Seven genotype categories have been proposed for HBV based on nucleotide divergence within the genome of greater than 8%.[35,35] These genotypes are designated with capital alphabetical letters from A through G. The HBV genotypes have characteristic geographic distributions.[36]

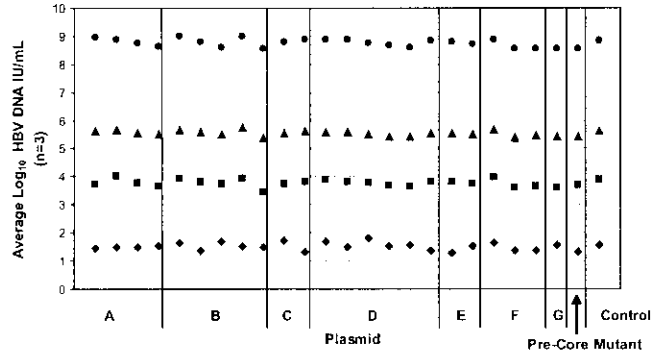
The performance of the COBAS® TaqMan® HBV Test on HBV genotypes was evaluated by analysis of 23 purified, linearized and quantitated plasmid DNAs containing representative sequence inserts from HBV genotypes A through G (*see* Table 1). In addition, a plasmid representing the common pre-Core mutation G:A at nucleotide position 1896 was tested. Each plasmid DNA was diluted to concentrations of 5.2E1, 5.2E2, 5.2E5 and 5.2E8 IU/mL. Each dilution was co-amplified with HBV QS DNA and analyzed in triplicate with the COBAS® TaqMan® HBV Test. The titers for all plasmids were compared with that of a control plasmid DNA (pc).

The COBAS® TaqMan® HBV Test gave equivalent results for all 23 plasmid DNAs and the pre-Core mutation. The copy numbers for all genotypes and the pre-Core mutation were in good agreement with each other and the control plasmid DNA.

Table 1
COBAS® TaqMan® HBV Test Inclusivity Testing — Typed Plasmid DNA Tested

Plasmid Designation	Genotype	Parent Specimen Origin
p8423-c1	A	India
p1115-c1	A	Burundi
p3952-c1	A	Cameroon
p4199-c2	A	Norway
p1764-c1	B	China
p1767-c1	B	China
p3958-c1	B	East Asia
p830-c1	B	Societe Island
p3982-c1	B	Vietnam
p1786-c1	C	China
p11549-1	C	Bangladesh
p3872-c1	D	Iran
p1103-c1	D	Tunisia
p3953-c2	D	North Africa
p18-c1	D	Sweden
p30893-5	D	Sweden
p4244-c1	D	Denmark
p3217-c1	E	Senegal
p3963-c2	E	Nigeria
p9203 c1	F	Colombia
p479-c1	F	Venezuela
p1009-c1	F	Spain
p00042975-4	G	United States
pFT1896	Pre-Core	Italy

Figure 10
Performance of the COBAS® TaqMan® HBV Test on HBV Genotypes A through G and a Pre-Core Mutant



Limit of Detection Using the WHO International Standard

The limit of detection was determined using the WHO International Standard in accordance with the methods defined in the CLSI (formerly NCCLS) Guideline EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline."[46] The WHO Standard was freshly diluted into each of five unique EDTA plasma specimens and each of five unique serum specimens. Each level of each dilution was tested with six replicates split across two runs for each of two reagent lots for each matrix. A total of 10 runs were conducted over five days for each reagent lot for each matrix to give a total of 60 replicates for each level for each matrix. These studies demonstrate that the COBAS® TaqMan® HBV Test can detect HBV DNA in EDTA plasma and serum at concentrations as low as 10 IU/mL with a positivity rate greater than 95%. The concentration of HBV DNA using the WHO International Standard in EDTA plasma and serum that can be detected with a positivity rate of greater than 95% as determined by Probit Analysis, is 3.5 IU/mL and 3.4 IU/mL, respectively (*see* Table 2 and Table 3).

Table 2
Limit of Detection in EDTA Plasma of the COBAS® TaqMan® HBV Test
For Use With The High Pure System using the WHO International Standard (Genotype A)

WHO Standard Based Concentration (HBV DNA IU/mL)	No. Valid Replicates	No. Positives	Positivity Rate
29.0	59	59	100%
22.0	59	59	100%
15.0	60	60	100%
12.0	59	59	100%
10.0	59	59	100%
8.0	60	59	98%
6.0	60	59	98%
4.0	60	59	98%
2.0	60	51	85%
1.0	60	37	62%
0.5	60	23	38%
0.0	60	0	0%
Probit 95% Hit Rate	3.5 IU/mL [95% confidence limits of 2.8 – 4.7 IU/mL]		

Table 3
Limit of Detection in Serum of the COBAS® TaqMan® HBV Test
For Use With The High Pure System using the WHO International Standard (Genotype A)

WHO Standard Based Concentration (HBV DNA IU/mL)	No. Valid Replicates	No. Positives	Positivity Rate
29.0	59	59	100%
22.0	59	59	100%
15.0	60	60	100%
12.0	60	60	100%
10.0	59	59	100%
8.0	59	58	98%
6.0	60	59	98%
4.0	60	58	97%
2.0	59	52	88%
1.0	59	39	66%
0.5	60	19	32%
0.0	60	0	0%
Probit 95% Hit Rate	3.4 IU/mL [95% confidence limits of 2.7 4.6 IU/mL]		

Limit of Detection Using Clinical Specimens Across All HBV Genotypes

The LOD was determined using two lots of reagents for seven clinical specimens of HBV representing genotypes A through G diluted into both EDTA plasma and serum. One representative clinical specimen of each genotype was tested. The HBV titer for each parent specimen was provided by the vendor or determined in-house. Dilution panels were generated from these final titer assignments. Each panel consisted of six members representing input levels at 15, 10, 8, 6, 4 and 1 IU/mL. Each level of each dilution was tested with 16 replicates split across two runs for each of two reagent lots for each genotype specimen in each matrix across eight days. A total of 32 replicates of each panel member was tested for each genotype in each matrix. The hit rate was determined at each input level and the Limit of Detection is defined as the lowest level demonstrating a ≥ 95% hit rate and where all higher input levels have ≥ 95% hit rate. The limit of detection for the various genotypes in EDTA plasma and serum is summarized in Table 4 below. The data shown represents the combined results from testing with two lots of reagents.

Table 4
LOD of HBV Genotypes

Genotype	EDTA Plasma		Serum	
	LOD, IU/mL	Hit Rate (95% Confidence Limits)	LOD, IU/mL	Hit Rate (95% Confidence Limits)
A	10	100% (89.1 – 100%)	4	100% (89.1 – 100%)
B	4	97% (83.8 – 99.9%)	4	100% (89.1 – 100%)
C	4	97% (83.8 - 99.9%)	4	100% (89.1 – 100%)
D	4	100% (89.1 – 100%)	4	97% (83.8 – 99.9%)
E	4	97% (83.8 – 99.9%)	4	100% (89.1 – 100%)
F	4	100% (89.1 – 100%)	4	100% (89.1 – 100%)
G	6	97% (83.3 – 99.9%)	4	100% (89.1 – 100%)

The LOD for the COBAS® TaqMan® HBV Test, detecting any of the seven genotypes tested, is determined to be 10 IU/mL. No significant difference between the seven genotypes was observed. In addition, there was no significant difference between plasma and serum or between the two lots of reagents tested.

Considering that the COBAS® TaqMan® HBV Test does not differentiate between HBV genotypes, the overall LoD of the assay to detect HBV in clinical specimens is determined to be 10 IU/mL.

Limit of Quantitation

The limit of quantitation was determined using the WHO International Standard in accordance with the methods defined in the CLSI Guideline EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline."[16] The WHO Standard was freshly diluted into each of five unique EDTA plasma specimens and each of five unique serum specimens. Each level of each dilution was tested with six replicates split across two runs for each of two reagent lots for each matrix. A total of 10 runs were conducted across five days for each reagent lot for each matrix to give a total of 60 replicates for each level for each matrix. As demonstrated in Table 5, the studies demonstrated that the COBAS® TaqMan® HBV Test can determine the concentration of HBV DNA in EDTA plasma and serum at concentrations as low as 29 IU/mL with an acceptable level of accuracy.

Table 5
Limit of Quantitation

Matrix	Expected Concentration	Expected $\log_{10}$ Concentration	Observed Avg. $\log_{10}$ Concentration	Absolute Bias	SD $\log_{10}$ Concentration	Total Analytical Error[1]
EDTA Plasma	29 IU/mL	1.462	1.25	0.21	0.24	0.69
Serum	29 IU/mL	1.462	1.247	0.22	0.17	0.56

[1] *The Total Analytical Error, or TAE, is defined as Bias + 2SD; the TAE was 0.69 for plasma and 0.56 for serum. At this concentration, the difference between two measurements of more than 1.0 $\log_{10}$ IU/mL is statistically significant.*

Precision

Within-laboratory Precision

Within-Run, Run-to-Run and Total Precision were evaluated in accordance with the methods defined in the CLSI (formerly NCCLS) Guideline EP5-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline–Second Edition."[33] This procedure permits the determination of both Within-Run and Total Precision through the performance of a single multiple-day and multiple operator study. A run, consisting of three replicates of each of ten panel members diluted from an HBV genotype A clinical specimen, was performed daily for 15 days. Each panel member was taken through the entire COBAS® TaqMan® HBV Test procedure, including specimen preparation, amplification and detection. Therefore, the precision reported here represents all aspects of the test procedure. The study was performed for three lots of COBAS® TaqMan® HBV Test reagents, and the combined results are shown in Table 6 (HBV DNA IU/mL) and Table 7 (HBV DNA $\log_{10}$ IU/mL).

Table 6
Precision of the COBAS® TaqMan® HBV Test (in IU/mL)

Specimen	1	2	3	4	5	6	7	8	9	10
Average Observed HBV DNA Titer (IU/mL)	1.07E8	5.33E7	1.04E7	8.52E5	9.28E4	1.21E4	1200	111	49	14
Within-Run CV	19%	10%	12%	7%	14%	16%	14%	22%	27%	50%
Run-to-Run CV	11%	14%	12%	14%	16%	18%	18%	26%	17%	22%
Total CV	23%	17%	17%	16%	22%	24%	22%	34%	32%	54%
Total No. Replicates	132	134	134	135	135	134	135	135	135	135

Table 7
Precision of the COBAS® TaqMan® HBV Test (in log_{10} IU/mL)

Specimen	1	2	3	4	5	6	7	8	9	10
Average Observed HBV DNA Titer ($\log_{10}$ IU/mL)	8.02	7.72	7.01	5.92	4.94	4.06	3.07	2.03	1.67	1.05
Within-Run Standard Deviation	0.07	0.04	0.05	0.03	0.21	0.18	0.06	0.09	0.11	0.59
Run-to-Run Standard Deviation	0.05	0.06	0.05	0.06	0.07	0.07	0.08	0.10	0.07	0.00
Total Standard Deviation	0.08	0.07	0.07	0.07	0.22	0.19	0.10	0.13	0.13	0.59

Reproducibility

The reproducibility of the COBAS® TaqMan® HBV Test For Use With the High Pure System was evaluated by two operators at each of three external clinical sites. Each operator performed three days of testing on each of three lots of reagents with each panel. Each run comprised a single panel with each panel member tested in triplicate.

The results of the reproducibility study are summarized in Table 8 to Table 10 (EDTA plasma) and Table 11 to Table 13 (serum).

Table 8
Components of Variance (Percentage of Total Variance) of HBV DNA Concentration (log_{10} IU/mL) — EDTA Plasma

Genotype	Mean of HBV DNA Concentration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/ Instrument	Operator	Day/Run	Within-Run	Total Precision Variance of log_{10} HBV DNA Concentration
A	1.32	21	162[a]	0.0015 (3%)	0.0000 (0%)	0.0008 (1%)	0.0044 (7%)	0.0521 (89%)	0.0588 (100%)
	2.34	220	162	0.0004 (5%)	0.0000 (0%)	0.0008 (11%)	0.0015 (20%)	0.0048 (64%)	0.0074 (100%)
	3.36	2,314	162	0.0003 (4%)	0.0000 (0%)	0.0019 (30%)	0.0014 (22%)	0.0027 (44%)	0.0062 (100%)
	4.35	22,369	162	0.0010 (12%)	0.0002 (2%)	0.0019 (24%)	0.0019 (23%)	0.0032 (39%)	0.0081 (100%)
	5.19	154,752	162	0.0014 (24%)	0.0003 (5%)	0.0011 (20%)	0.0011 (20%)	0.0017 (31%)	0.0056 (100%)
	7.29	19,444,058	162	0.0000 (1%)	0.0000 (0%)	0.0005 (14%)	0.0007 (21%)	0.0022 (64%)	0.0035 (100%)
C	1.38	24	162	0.0020 (9%)	0.0006 (3%)	0.0006 (3%)	0.0059 (25%)	0.0143 (61%)	0.0235 (100%)
	2.34	219	160[b]	0.0022 (34%)	0.0004 (6%)	0.0005 (7%)	0.0000 (0%)	0.0035 (54%)	0.0064 (100%)
	3.43	2,686	162	0.0004 (7%)	0.0016 (29%)	0.0009 (17%)	0.0008 (15%)	0.0019 (33%)	0.0057 (100%)
	4.39	24,479	161[c]	0.0004 (8%)	0.0010 (21%)	0.0008 (16%)	0.0011 (22%)	0.0015 (32%)	0.0047 (100%)
	5.24	172,515	162	0.0007 (17%)	0.0009 (20%)	0.0008 (19%)	0.0006 (13%)	0.0014 (31%)	0.0044 (100%)
	7.38	23,791,720	162	0.0013 (21%)	0.0001 (1%)	0.0004 (8%)	0.0008 (14%)	0.0033 (56%)	0.0059 (100%)

[a] *14 out of 162 (8.6%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid.*

Table 9
Standard Deviation Components HBV DNA Concentration (log_{10} IU/mL) — EDTA Plasma

Geno-type	Mean of HBV DNA Concen-tration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/ Instru-ment	Operator	Day/ Run	Within-Run	Total Standard Deviation of log_{10} HBV DNA Concentration
A	1.32	21	162[a]	0.0391	0.0000	0.0277	0.0662	0.2283	0.2425
	2.34	220	162	0.0188	0.0000	0.0291	0.0381	0.0691	0.0860
	3.36	2,314	162	0.0161	0.0000	0.0431	0.0368	0.0522	0.0787
	4.35	22,369	162	0.0311	0.0128	0.0441	0.0431	0.0564	0.0900
	5.19	154,752	162	0.0368	0.0161	0.0329	0.0337	0.0416	0.0748
	7.29	19,444,058	162	0.0051	0.0000	0.0222	0.0268	0.0474	0.0592
C	1.38	24	162	0.0449	0.0251	0.0246	0.0770	0.1196	0.1533
	2.34	219	160[b]	0.0466	0.0190	0.0212	0.0000	0.0589	0.0800
	3.43	2,686	162	0.0205	0.0402	0.0307	0.0286	0.0431	0.0755
	4.39	24,479	161[c]	0.0196	0.0319	0.0274	0.0327	0.0391	0.0686
	5.24	172,515	162	0.0270	0.0292	0.0290	0.0242	0.0368	0.0663
	7.38	23,791,720	162	0.0354	0.0079	0.0212	0.0285	0.0574	0.0768

[a] *14 out of 162 (8.6%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid.*

Table 10
Reproducibility Results Summary:
Total %CV for HBV Panel Members — EDTA Plasma

Genotype	Mean of HBV DNA Concentration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Total Precision Variance of log_{10} HBV DNA Concentration	Total Precision Standard Deviation of log_{10} HBV DNA Concentration	lognormal CV (%)
A	1.32	21	162[a]	.0588	0.24	60
	2.34	220	162	.0074	0.09	20
	3.36	2,314	162	.0062	0.08	18
	4.35	22,369	162	.0081	0.09	21
	5.19	154,752	162	.0056	0.07	17
	7.29	19,444,058	162	.0035	0.06	14
C	1.38	24	162	.0235	0.15	36
	2.34	219	160[b]	.0064	0.08	19
	3.43	2,686	162	.0057	0.08	17
	4.39	24,479	161[c]	.0047	0.07	16
	5.24	172,515	162	.0044	0.07	15
	7.38	23,791,720	162	.0059	0.08	18

[a] *14 out of 162 (8.6%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid.*

Table 11
Components of Variance (Percentage of Total Variance) of HBV DNA Concentration (log_{10} IU/mL) — Serum

Geno-type	Mean of HBV DNA Concen-tration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/ Instru-ment	Operator	Day/Run	Within-Run	Total Precision Variance of log_{10} HBV DNA Concentration
A	1.04	11	162[a]	0.0054 (5%)	0.0127 (13%)	0.0010 (1%)	0.0044 (4%)	0.0767 (77%)	0.1001 (100%)
	2.10	127	160[b]	0.0000 (0%)	0.0022 (16%)	0.0007 (5%)	0.0043 (30%)	0.0072 (50%)	0.0144 (100%)
	3.34	2,194	162	0.0000 (1%)	0.0022 (28%)	0.0009 (11%)	0.0016 (21%)	0.0031 (39%)	0.0078 (100%)
	4.34	21,749	161[c]	0.0020 (20%)	0.0029 (29%)	0.0008 (8%)	0.0023 (23%)	0.0019 (19%)	0.0099 (100%)
	5.17	147,146	162	0.0024 (35%)	0.0011 (16%)	0.0007 (10%)	0.0016 (24%)	0.0011 (16%)	0.0069 (100%)
	7.27	18,732,744	162	0.0000 (0%)	0.0003 (6%)	0.0001 (4%)	0.0003 (7%)	0.0033 (83%)	0.0039 (100%)
C	1.38	24	162	0.0005 (2%)	0.0000 (0%)	0.0032 (9%)	0.0068 (19%)	0.0244 (70%)	0.0349 (100%)
	2.34	218	161[c]	0.0010 (11%)	0.0000 (0%)	0.0005 (5%)	0.0040 (48%)	0.0030 (35%)	0.0084 (100%)
	3.43	2,664	162	0.0003 (5%)	0.0003 (5%)	0.0009 (15%)	0.0029 (46%)	0.0018 (29%)	0.0062 (100%)
	4.39	24,555	162	0.0009 (12%)	0.0004 (6%)	0.0005 (6%)	0.0040 (53%)	0.0018 (24%)	0.0076 (100%)
	5.22	167,232	162	0.0009 (13%)	0.0006 (9%)	0.0005 (7%)	0.0032 (46%)	0.0017 (24%)	0.0070 (100%)
	7.37	23,676,552	161[c]	0.0006 (8%)	0.0000 (0%)	0.0004 (5%)	0.0041 (51%)	0.0030 (37%)	0.0082 (100%)

[a] *65 out of 162 (40.1%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid.*

Table 12
Standard Deviation Components HBV DNA Concentration (log_{10} IU/mL) — Serum

Genotype	Mean of HBV DNA Concentration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/ Instrument	Operator	Day/Run	Within-Run	Total Standard Deviation of log_{10} HBV DNA Concentration
A	1.04	11	162[a]	0.0737	0.1127	0.0312	0.0660	0.2769	0.3164
	2.10	127	160[b]	0.0000	0.0473	0.0260	0.0654	0.0850	0.1200
	3.34	2,194	162	0.0067	0.0470	0.0292	0.0406	0.0553	0.0883
	4.34	21,749	161[c]	0.0451	0.0539	0.0281	0.0482	0.0435	0.0995
	5.17	147,146	162	0.0489	0.0332	0.0262	0.0403	0.0328	0.0831
	7.27	18,732,744	162	0.0000	0.0160	0.0121	0.0164	0.0573	0.0624
C	1.38	24	162	0.0232	0.0000	0.0566	0.0823	0.1562	0.1868
	2.34	218	161[c]	0.0309	0.0000	0.0215	0.0636	0.0547	0.0917
	3.43	2,664	162	0.0181	0.0179	0.0306	0.0536	0.0421	0.0787
	4.39	24,555	162	0.0297	0.0210	0.0214	0.0634	0.0424	0.0872
	5.22	167,232	162	0.0302	0.0251	0.0221	0.0569	0.0413	0.0837
	7.37	23,676,552	161[c]	0.0249	0.0000	0.0204	0.0644	0.0549	0.0906

[a] *65 out of 162 (40.1%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid.*

Table 13
Reproducibility Results Summary:
Total %CV for HBV Panel Members — Serum

Genotype	Mean of HBV DNA Concentration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Total Precision Variance of log_{10} HBV DNA Concentration	Total Precision Standard Deviation of log_{10} HBV DNA Concentration	lognormal CV (%)
A	1.04	11	162[a]	.1001	0.32	84
	2.10	127	160[b]	.0144	0.12	28
	3.34	2,194	162	.0078	0.09	21
	4.34	21,749	161[c]	.0099	0.10	23
	5.17	147,146	162	.0069	0.08	19
	7.27	18,732,744	162	.0039	0.06	15
C	1.39	24	162	.0349	0.19	45
	2.34	218	161[c]	.0084	0.09	21
	3.43	2,664	162	.0062	0.08	18
	4.39	24,555	162	.0076	0.09	20
	5.22	167,232	162	.0070	0.08	19
	7.37	23,676,552	161[c]	.0082	0.09	21

[a] *65 out of 162 (40.1%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid*

Table 14 summarizes the results for HBV Negative Panel Members from the reproducibility study for each matrix. Eleven false positives were observed in the study and all were below the LOD. Specificity was 100% in EDTA plasma [95% CI = (0.98, 1.00)] and 97% in serum [95% CI = (0.94, 0.99)].

Table 14
HBV Negative Panel Member Summary

Matrix	Total Valid Results	Target Not Detected	Target Detected but Below LOD[1]	≥ 10 and < 29 IU/mL[2]	Within Linear Range[3]
EDTA Plasma	324	323	1	0	0
Serum	322	312	10	0	0

[1] *The limit of detection (LOD) for the assay is 10 IU/mL. Results < 10 IU/mL are below the LOD.*
[2] *Results ≥ 10 IU/mL to < 29 IU/mL are above the LOD, but below the linear range.*
[3] *Linear range (>29 IU/mL to 1.10E+8 IU/mL).*

Performance of COBAS® TaqMan® HBV Test with HBV-Negative Samples

The performance of the COBAS® TaqMan® HBV Test with HBV-negative samples was determined by analysis of HBV-negative serum and EDTA plasma from blood donors. A total of 220 specimens (110 individual EDTA plasma and 110 serum specimens) that were non-reactive for HBsAg and anti-HBc were tested. All specimens were noted as Target Not Detected for HBV DNA for both EDTA plasma and serum (100%, or 110/110, with a 95% confidence interval of 96.6% to 100%).

Analytical Specificity

Cross-reactivity

The analytical specificity of the COBAS® TaqMan® HBV Test was evaluated by adding cultured virus into HBV-negative human EDTA plasma or analyzing specimens from subjects positive for other viral agents (listed in Table 15 below). Except for one CMV infected specimen and HPV strain 18, none of the non-HBV DNA or RNA viruses tested were positive for HBV DNA. Subsequent testing of the CMV infected specimen did not consistently confirm the initial result. Subsequent testing of HPV strain 18 indicated that no positive results for HBV were detected at HPV concentrations less than 2.0E+09 cp/mL.

Table 15
Analytical Specificity Specimens

Virus Added Into Plasma	Specimens from Infected Patients (n)
Adenovirus type 7	Cytomegalovirus infected patients (2)[2]
Cytomegalovirus AD-169	Epstein-Barr Virus infected patients (2)
Epstein-Barr Virus (RAJI Burkitt's Lymphoma cells)	Hepatitis A Virus infected patients (2)
Hepatitis A Virus PA21	Hepatitis C Virus infected patient genotype 4 (1)
Herpes Simplex type 1, MacIntyre	Hepatitis C Virus infected patient genotype 6a (1)
Herpes Simples type 2, MS	HIV-1 infected patients (2)
Human Papilloma Virus Strain 18[1]	
Influenza A virus A/Hong Kong/8/68	
Influenza B virus B/R75	
Varicella-Zoster Ellen	
West Nile Virus	

[1] *HPV strain 18 returned a positive HBV result at an HPV concentration of 2.0E+09 cp/mL. Subsequent testing indicated no HBV positive results for HPV concentrations less than 2.0E+09 cp/mL.*
[2] *One of the two CMV specimens from infected patients returned a positive result which was not consistently confirmed in subsequent testing.*

Interfering Substances

Clinical specimens with elevated levels of triglycerides, bilirubin, albumin and hemoglobin were tested in the absence and presence (approximately 150 IU/mL) of HBV and have been shown not to interfere with the quantitation of HBV DNA by this test.

	Range of Specimens Tested	Normal Range
Triglycerides	655 – 1,378 mg/dL	45 – 190 mg/dL
Bilirubin	3.7 – 8.2 mg/dL	0.25 – 1.2 mg/dL
Albumin	5,100 – 6,600 mg/dL	2,800 – 5,000 mg/dL
Hemoglobin	27.5 – 243.2 mg/dL	0 – 2.5 mg/dL

The following drug compounds were tested at $1xC_{max}$ and $3xC_{max}$ for each drug in the absence and presence (approximately 150 IU/mL) of HBV and have been shown not to interfere with the quantitation of HBV DNA by this test.

Nucleotide DNA Polymerase Inhibitors Adefovir dipivoxil Tenofovir disoproxil fumarate	**Nucleoside Reverse Transcriptase and DNA Polymerase Inhibitors** Lamivudine Zidovudine Zalcitabine Stavudine Abacavir
HIV Protease Inhibitors Indinavir Ritonavir Nelfinavir Saquinavir Amprenavir Lopinavir/Ritonavir	**Non-nucleoside HIV Reverse Transcriptase Inhibitors** Nevirapine Efavirenz
Immune Modulators Interferon alpha-2a Interferon alpha-2b	**HIV Fusion Inhibitor** Enfuvirtide
CMV Treatment Compounds Ganciclovir Valganciclovir hydrochloride Acyclovir Valacyclovir hydrochloride	

Matrix Equivalency — Serum versus EDTA Plasma

Sixty matched clinical specimen sets (each set is EDTA plasma and serum drawn from a single HBV-infected or HBsAg-positive individual) were tested to demonstrate plasma – serum equivalency. Each sample was tested in duplicate and the mean titer for each sample was calculated. A Deming linear regression analysis was also performed using the calculated mean titers. Fifty matched sets were used for data analysis which included 41 matched sets testing within the linear range of the test and nine matched sets with titers above the linear range of the assay upon initial testing and retested following 1:100 dilution to obtain the titer of the original sample. The results from ten sets (five negative for HBV DNA and five sets positive for HBV DNA but with titers too low to quantify) were not included in the final analysis.

The individual log titer difference (log titer EDTA plasma – log titer serum) for 49 of the 50 matched sets was ≤ 0.30 with the one set having a difference of -0.37. The mean difference was -0.05 log (95% CI: -0.036, 0.025), indicating that the results between serum and EDTA plasma were not significantly different. The result from the linear regression analysis is shown in Figure 11 slope=1.0131 (95% confidence interval is [1.0045 – 1.0216]) with an intercept of -0.0605 (95% confidence interval is [-0.1065 to -0.0144]). The pooled standard deviation estimates for the EDTA plasma and serum samples are shown in Table 16.

Figure 11
Regression Analysis of Matched Serum — EDTA Plasma Samples (n=50)

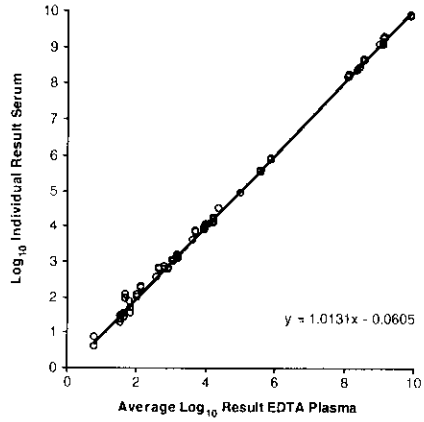


Table 16
Pooled Standard Deviation Estimates for
EDTA Plasma and Serum Specimens in the Matrix Equivalency Study

Matrix	Mean log Titer	Pooled SD
EDTA Plasma	4.621	0.060
Serum	4.616	0.093

CLINICAL PERFORMANCE

Literature Review of HBV DNA in Clinical Practice

Guidelines published in the medical literature support the importance of measuring HBV levels at baseline prior to treatment, at intervals during treatment to monitor antiviral response as well as at intervals on therapy to survey for the development of drug resistance.[37,44] The American Association for the Study of Liver Disease (AASLD) guidelines from 2007 make specific recommendations for initiating HBV therapy based on the level of pre-treatment serum ALT, HBeAg status and level of HBV DNA. In these guidelines, HBV DNA values of > 20,000 IU/mL and > 2,000 IU/mL for chronic hepatitis B (CHB) and cirrhosis respectively were selected as the level at which to initiate treatment.[44] The threshold level of HBV DNA for determination of candidacy differs in other published guidelines (≥ 20,000 IU/mL or ~10^5 copies/mL for patients with HBeAg-positive CHB; ≥ 2,000 IU/mL or ~10^4 copies/mL for patients with HBeAg-negative CHB; and ≥ 200 IU/mL or ~10^3copies/mL for patients with decompensated cirrhosis).[37]

Treatment goals have evolved over time although goals are consistent across recently published guidelines.[37,38,44] Hoofnagle et al state that the major goals of therapy are not immediate amelioration of symptoms (since CHB is typically silent), but rather long-term prevention of progression, development of cirrhosis and hepatocellular carcinoma (HCC).[38] Improvement in histology, a surrogate for improvement in natural history, has been a primary efficacy end-point in the design of clinical trials that led to FDA-approval of lamivudine and adefovir.[34,39,40] Other end-points include HBeAg seroconversion in HBeAg positive patients, normalization of ALT and undetectable or reduced HBV DNA. Loss of HBsAg is the most desirable end-point of treatment, but this is rarely achieved with nucleoside analogs.[38] The AASLD[44] guidelines state that the aims of treatment of chronic hepatitis B are to achieve sustained suppression of HBV replication and remission of liver disease. Similarly, the guideline of Keeffe et al[37] states that the goal of therapy for CHB should be

to significantly suppress HBV replication and prevent clinical progression of liver disease. Hoofnagle et al state that virological responses based upon testing for levels of HBV DNA in serum are probably the most appropriate criteria for assessing outcome of antiviral therapy.[38] Hence, a goal of treatment is to reduce and maintain serum HBV DNA at the lowest possible levels (durable HBV DNA suppression). Viral suppression was also the primary goal of therapy in the Asian Pacific Association for the Study of the Liver (APASL) guidelines.[41] According to Hoofnagle et al, virological response is defined as the lack of detectable HBV DNA in serum using an assay that is sensitive to 20-100 IU/mL (~100-500 copies/mL).[38] Viral suppression will lead to other goals of therapy, including histological improvement, HBeAg loss and/or HBeAg seroconversion, and ALT normalization. In patients who are HBeAg-positive before therapy, an additional goal of treatment is loss of HBeAg with seroconversion to anti-HBe. The latter is preferable because attainment of complete HBeAg seroconversion indicates that antiviral therapy may be discontinued, and the likelihood is high that the benefit will persist off-therapy. Loss of HBsAg is rarely achieved with short-term antiviral therapy and therefore not a common goal for antiviral trials.

To measure durable viral suppression, Keeffe et al, recommend that patients be monitored at least every six months while on therapy with either entecavir or adefovir, and more frequently with lamivudine to identify resistance.[37] Patients should be treated after HBeAg seroconversion as long as HBV DNA levels are decreasing until there are undetectable HBV DNA levels by PCR.[37] Treatment should then be continued for an additional six to twelve months. In patients who have demonstrated HBeAg seroconversion but in whom HBV DNA levels are detectable and stable, treatment should be continued for six months; seroconversion should be documented again, then consideration given to stopping treatment (in noncirrhotic patients). It should be noted that these recommendations reflect the opinions of the expert panel that convened this treatment algorithm, and although not always based on randomized controlled trials, are aimed at ultimately reducing the severity of liver disease.

During treatment, HBV DNA levels can also serve as a surrogate for identifying viral resistance.[36,44] Risk for development of resistance differs between FDA-approved therapies. Development of resistance is associated with HBV DNA rebound, followed by ALT elevation, reversal of histological improvement and, in some cases, progressive liver disease associated with severe exacerbations.[37]

Overall, the goal of therapy for patients with chronic HBV infection is to prevent progression of liver disease to cirrhosis and HCC. Because HBV replication is implicated in these outcomes, the primary aim of therapy is durable suppression of serum HBV DNA.[44] This goal requires sensitive HBV DNA tests with a broad dynamic range. Quantitative "real-time" PCR enables the accurate measuring of HBV DNA at low levels, to establish a patient's baseline HBV DNA prior to treatment, and to monitor response to antiviral therapy or viral rebound associated with resistance. The threshold level of HBV DNA to initiate treatment differs in different populations, ranging from 2,000 IU/mL for patients with cirrhosis to 20,000 IU/mL for patients with chronic HBV disease. As previously discussed, HBV DNA testing is integral to many aspects of the management of patients with chronic HBV infection, and is critical for assessing the response to antiviral therapy, along with other laboratory and clinical considerations.

Clinical Performance

The clinical performance of the COBAS® TaqMan® HBV Test For Use With The High Pure System was evaluated by assessing the antiviral therapy response in chronic HBV-infected subjects undergoing treatment with adefovir dipivoxil. Assessment was performed at Screening and at Weeks 4, 8, 16, 28, 44, and 48 (when available). The primary objective was to determine the relationship between viral levels at various treatment time points compared with histological, serological, and biochemical responses to treatment.

The results from testing with the COBAS® TaqMan® HBV Test For Use With The High Pure System were used to determine whether change (or absence of change) in HBV viral load at various time points may predict improvement (or lack of improvement) in a patient's immune response marker or liver histology at different treatment time points. Statistical analysis of clinical data was used to assess whether viral response to treatment measured with COBAS® TaqMan® HBV Test For Use With the High Pure System is informative for assessing the response to treatment in HBeAg+ and HBeAg- patients with chronic hepatitis B. Observing changes in viral load in individual patients over time may help the clinician in the assessment of a patient's response to therapy.

Study Population

The HBV DNA data were obtained from testing of patient samples previously collected under two study protocols, one of which evaluated patients with chronic HBeAg+ HBV infection and compensated liver function and one that evaluated patients with presumed precore mutant (HBeAg-/ HBV DNA+) chronic HBV infection with compensated liver function. Marcellin et el.[34] and Hadziyannis et al.[40] previously described subject selection for both studies.

The study population consisted of 407 chronic HBV infected patients enrolled in double-blind, randomized, placebo-controlled studies of Adefovir Dipivoxil. Demographic data, drug dosing data, HBV genotype, HBeAg and anti-HBe results, ALT results, and Baseline (pre-treatment) and end-point liver biopsy results were available for each patient. Viral load testing was performed at Screening and at Weeks 4, 8, 16, 28, 44, and 48 (when available).

Table 17 below summarizes the study population at Screening.

Table 17
Description of Study Population at Screening

Characteristic	Category	Summary Statistics	HBeAg+	HBeAg-	Total
Total Number of Subjects	—	N	264	143	407
• Placebo	—	n (%)	129 (49)	50 (35)	179 (44)
• 10 mg Adefovir Dipivoxil		n (%)	135 (51)	93 (65)	228 (56)
Age (yr)		Median (Min, Max)	34 (16, 65)	46 (18, 65)	39 (16, 65)
Weight (kg)	—	Median (Min, Max)	70 (41, 118)	74 (46, 111)	72 (41, 118)
Sex	Male	N (%)	191 (72)	119 (83)	310 (76)
	Female	N (%)	73 (28)	24 (17)	97 (24)
Race	White	N (%)	86 (33)	90 (63)	176 (43)
	Asian	N (%)	167 (63)	48 (34)	215 (53)
	Other	N (%)	11 (4)	5 (3)	16 (4)
Genotype	A	N (%)	73 (28)	9 (6)	82 (20)
	B	N (%)	49 (19)	28 (20)	77 (19)
	C	N (%)	111 (42)	19 (13)	130 (32)
	D	N (%)	25 (9)	84 (59)	109 (27)
	Other	N (%)	6 (2)	3 (2)	9 (2)
HBV DNA < 1.72E+04 IU/mL	—	N (%)	3 (1)	5 (3)	8 (2)
ALT ≤ ULN[1]		N (%)	5 (2)	7 (5)	12 (3)
Knodell Score	—	N	259	139	398
Total	—	Mean (SD)	9.5 (3.3)	9.4 (3.4)	9.5 (3.3)
Necroinflammatory	—	Mean (SD)	7.7 (2.8)	7.5 (2.8)	7.6 (2.7)
Fibrosis	—	Mean (SD)	1.7 (1.1)	1.9 (1.2)	1.8 (1.1)

[1] *ULN = Upper Limit of Normal Range*

Screening samples were obtained six to 125 days before study start. On average, HBeAg+ patients were younger than the HBeAg- patients (median age = 34 years vs 46 years), predominantly Asian (63% vs 34%), were female (28% vs 17%) and were infected with primarily HBV genotypes A and C (70% vs 19%). HBeAg- patients were predominantly White (63%) and infected with HBV genotype D (59%). The Knodell necropsy scores for necroinflammation and fibrosis at Baseline (pre-treatment) were comparable for both populations.

Patients included in the clinical performance analysis received either the standard 10 mg Adefovir dipivoxil dosing or placebo, as indicated in Table 18.

Table 18

Summary of Available Samples by Treatment Arm

Population	No. Subjects — Placebo	No. Subjects — 10 mg Adefovir	No. Samples per Subject	Total No. Samples
Chronic HBeAg+	129	135	7	1848
Chronic HBeAg-	50	93	6	858
Grand Total				**2706**

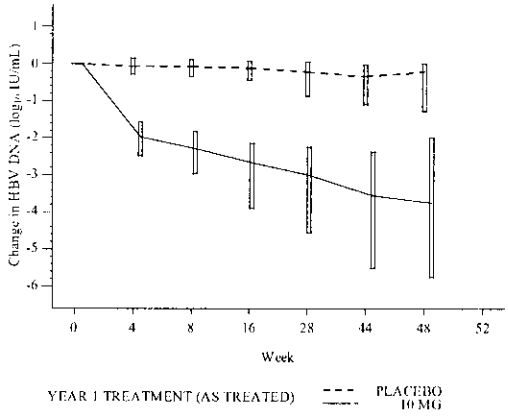
Within-Subject Variability in Absence of Treatment

The objective of this analysis was to estimate the change in viral load (in $\log_{10}$ IU/mL units) between two successive measurements of placebo patients. One hundred and seventy nine subjects were enrolled into the placebo arms of the HBeAg+ and HBeAg- studies of which 137 of 179 had results within the linear range of the assay for testing at Weeks 0, 4, and 8. These results were used to estimate within subject variability, which includes biological variability as well as total assay variability. The within subject variability from these results was estimated to be 0.58 $\log_{10}$ IU/mL for HBeAg- patients and 0.78 $\log_{10}$ IU/mL for HBeAg+ patients. Biological variability was similar to the within-subject variability since the assay variability was negligible. The median change of viral load within a subject was estimated to be 0.30 $\log_{10}$ IU/mL for HBeAg+ and 0.58 $\log_{10}$ IU/mL for HBeAg- patients. Approximately 90% of the HBeAg+ patients' and 80% of the HBeAg- patients' change of viral load was less than 2.0 $\log_{10}$ IU/mL.

Clinical Performance of the COBAS® TaqMan® HBV Test in HBeAg+ Patients on Therapy

A graph illustrating the change of the median viral load in patients on adefovir dipivoxil and on placebo is given in Figure 12. It demonstrates efficacy of treatment of the HBeAg+ patients with chronic hepatitis B with adefovir dipivoxil compared to placebo.

Figure 12
Median and Inter-Quartile Range of Change in HBV DNA from Screening: HBeAg+ Population



A useful tool for a clinician in monitoring treatment for HBeAg+ patients is to observe an HBV viral load increase of more than 1 $\log_{10}$ after reaching a nadir. Table 19 summarizes the data for the 135 HBeAg+ patients on adefovir dipivoxil treatment.

Table 19
Distribution of the HBeAg+ Patients by Week on Treatment and the Viral Load at Which the Nadir was Reached

Nadir Viral Load (IU/mL)	Number (%) of Patients With the Nadir Viral Load Achieved by Week						Total By Viral Load	Cumulative By Viral Load
	4	8	16	28	44	48		
TND*	0	0	0	0	1 (0.7)	0	1 (0.7)	1 (0.7)
< 10	0	0	2 (1.5)	3 (2.2)	7 (5.2)	6 (4.4)	18 (13.3)	19 (14.1)
10 – < 100	0	0	0	0	9 (6.7)	12 (8.9)	21 (15.6)	40 (29.6)
$100 - < 10^3$	0	0	2 (1.5)	1 (0.7)	0	10 (7.4)	13 (9.6)	53 (39.3)
$10^3 - < 10^4$	1 (0.7)	1 (0.7)	2 (1.5)	2 (1.5)	8 (5.9)	6 (4.4)	20 (14.8)	73 (54.1)
$10^4 - < 10^5$	0	0	1 (0.7)	5 (3.7)	3 (2.2)	10 (7.4)	19 (14.1)	92 (68.1)
$10^5 - < 10^6$	0	2 (1.5)	1 (0.7)	3 (2.2)	10 (7.4)	5 (3.7)	21 (15.6)	113 (83.7)
$\geq 10^6$	3 (2.2)	5 (3.7)	4 (3.0)	4 (3.0)	2 (1.5)	4 (3.0)	22 (16.3)	135 (100)
Total By Week	4 (3.0)	8 (5.9)	12 (8.9)	18 (13.3)	40 (29.6)	53 (39.3)		
Cumulative By Week	4 (3.0)	12 (8.9)	24 (17.8)	42 (31.1)	82 (60.7)	135 (100)		

* *TND = HBV DNA not detected.*

In this study, 60.7% (82/135) of the patients reached a nadir in viral load by week 44 on treatment. Seventeen patients had more than 1 $\log_{10}$ increase in viral load by week 48 after achieving the nadir, which is 12.6% (17/135) of the total number of patients on treatment and 20.7% (17/82) of the patients who achieved a nadir.

The results of the analysis of the associations between the responses to treatment at week 48 and the covariates are summarized in Table 20. There are no statistically significant associations – the lower limits of the 95% CIs of the odds ratios are smaller than 1 (0.26 - 0.96).

Table 20
Association Between Responses to Treatment at Week 48 and Baseline Covariates for HBeAg+ Patients

Response to Treatment	Covariate	Category	N	Number of Patients With Response	Proportion (%) of Patients With Response	Unadjusted Odds Ratio (95% CI)
Antigen Loss	Race	Asian	84	23	27.4	0.75 (0.32, 1.80)
		Other	45	15	33.3	
	Sex	Male	99	28	28.3	0.79 (0.31, 2.14)
		Female	30	10	33.3	
	Age	≤ 30	60	15	25.0	0.67 (0.28, 1.54)
		>30	69	23	33.3	
	Genotype	B,C	82	21	25.6	0.61 (0.26, 1.43)
		Non-B,C	47	17	36.2	
Histological	Race	Asian	79	52	65.8	1.20 (0.52, 2.69)
		Other	47	29	61.7	
	Sex	Male	95	65	68.4	2.03 (0.81, 5.02)
		Female	31	16	51.6	
	Age	≤ 30	57	39	68.4	1.39 (0.63, 3.13)
		>30	69	42	60.9	
	Genotype	B,C	77	52	67.5	1.43 (0.64, 3.21)
		Non-B,C	49	29	59.2	
Biochemical	Race	Asian	84	52	61.9	2.09 (0.96, 4.58)
		Other	48	21	43.8	
	Sex	Male	100	58	58.0	1.57 (0.65, 3.78)
		Female	32	15	46.9	
	Age	≤ 30	61	39	63.9	1.93 (0.91, 4.13)
		>30	71	34	47.9	
	Genotype	B,C	83	51	61.4	1.96 (0.90, 4.26)
		Non-B,C	49	22	44.9	

Further data analysis of the HBeAg+ population to demonstrate clinical performance of the COBAS® TaqMan® HBV Test was done using two different definitions of the early virological response to treatment: (1) HBV viral load < 2000 IU/mL (or approximately 10^4 cp/mL)[44], (2) a decrease in serum HBV DNA from an initial Screening viral load value by ≥ 2 $\log_{10}$.[44]

The statistical significance of the associations of the Race, Sex, Age and Genotype covariates with the viral response was studied. Odds ratios plus their exact 95% confidence intervals were calculated for both definitions of the viral response and summarized in Table 21 and Table 22. The logistic regression analysis of viral response as a function of the covariates showed no statistical significance of such associations for either definition of the viral response.

Table 21
Odds Ratios for the Association Between Viral Response (< 2000 IU/mL) and Covariates, by Week, for an HBeAg+ Population

Covariate	Category	Week	N	Number Below 2000 IU/mL	Proportion (%) Below 2000 IU/mL	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	86	4	4.7	0.75 (0.12, 5.34)
	Other		49	3	6.1	
	Asian	8	85	10	11.8	1.17 (0.34, 4.66)
	Other		49	5	10.2	
	Asian	16	84	20	23.8	2.19 (0.76, 7.18)
	Other		48	6	12.5	
	Asian	28	85	30	35.3	2.80 (1.10, 7.76)
	Other		49	8	16.3	
	Asian	44	83	36	43.4	1.81 (0.80, 4.20)
	Other		47	14	29.8	
	Asian	48	85	37	43.5	1.45 (0.66, 3.23)
	Other		49	17	34.7	
Sex	Male	4	102	3	2.9	0.22 (0.03, 1.40)
	Female		33	4	12.1	
	Male	8	101	11	10.9	0.89 (0.24, 4.11)
	Female		33	4	12.1	
	Male	16	100	20	20.0	1.08 (0.37, 3.65)
	Female		32	6	18.8	
	Male	28	101	27	26.7	0.73 (0.29, 1.90)
	Female		33	11	33.3	
	Male	44	98	38	38.8	1.06 (0.43, 2.66)
	Female		32	12	37.5	
	Male	48	101	40	39.6	0.89 (0.37, 2.16)
	Female		33	14	42.4	
Age	≤ 30	4	63	5	7.9	3.02 (0.47, 32.53)
	>30		72	2	2.8	
	≤ 30	8	62	6	9.7	0.75 (0.21, 2.54)
	>30		72	9	12.5	
	≤ 30	16	61	10	16.4	0.67 (0.25, 1.75)
	>30		71	16	22.5	
	≤ 30	28	62	16	25.8	0.79 (0.34, 1.80)
	>30		72	22	30.6	
	≤ 30	44	60	23	38.3	0.99 (0.46, 2.13)
	>30		70	27	38.6	
	≤ 30	48	62	23	37.1	0.78 (0.37, 1.65)
	>30		72	31	43.1	
Genotype	B,C	4	84	4	4.8	0.80 (0.13, 5.70)
	Non-B,C		51	3	5.9	
	B,C	8	83	10	12.0	1.26 (0.36, 5.00)
	Non-B,C		51	5	9.8	
	B,C	16	82	19	23.2	1.85 (0.67, 5.66)
	Non-B,C		50	7	14.0	
	B,C	28	83	28	33.7	2.09 (0.86, 5.35)
	Non-B,C		51	10	19.6	
	B,C	44	81	34	42.0	1.49 (0.67, 3.38)
	Non-B,C		49	16	32.7	
	B,C	48	83	35	42.2	1.23 (0.57, 2.69)
	Non-B,C		51	19	37.3	

Table 22
Odds Ratios for Association Between Viral Response
(≥2 log_{10} Decrease From Initial Screening Result) and Covariates, by Week, for HBeAg+ Population

Covariate	Category	Week	N	Number With ≥ 2-log_{10} Decrease	Proportion (%) With ≥ 2-log_{10} Decrease	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	85	46	54.1	2.43 (1.10, 5.45)
	Other		49	16	32.7	
	Asian	8	84	55	65.5	1.10 (0.49, 2.44)
	Other		49	31	63.3	
	Asian	16	83	66	79.5	1.02 (0.38, 2.65)
	Other		48	38	79.2	
	Asian	28	84	68	81.0	1.09 (0.40, 2.85)
	Other		49	39	79.6	
	Asian	44	82	66	80.5	1.11 (0.41, 2.93)
	Other		47	37	78.7	
	Asian	48	84	65	77.4	1.51 (0.63, 3.58)
	Other		49	34	69.4	
Sex	Male	4	101	44	43.6	0.64 (0.27, 1.53)
	Female		33	18	54.5	
	Male	8	100	62	62.0	0.61 (0.23, 1.55)
	Female		33	24	72.7	
	Male	16	99	77	77.8	0.65 (0.17, 2.00)
	Female		32	27	84.4	
	Male	28	100	80	80.0	0.89 (0.26, 2.62)
	Female		33	27	81.8	
	Male	44	97	77	79.4	0.89 (0.26, 2.63)
	Female		32	26	81.3	
	Male	48	100	74	74.0	0.91 (0.32, 2.42)
	Female		33	25	75.8	
Age	≤ 30	4	63	34	54.0	1.80 (0.86, 3.79)
	>30		71	28	39.4	
	≤ 30	8	62	42	67.7	1.29 (0.59, 2.82)
	>30		71	44	62.0	
	≤ 30	16	61	48	78.7	0.92 (0.36, 2.37)
	>30		70	56	80.0	
	≤ 30	28	62	50	80.6	1.02 (0.40, 2.67)
	>30		71	57	80.3	
	≤ 30	44	60	48	80.0	1.02 (0.39, 2.67)
	>30		69	55	79.7	
	≤ 30	48	62	48	77.4	1.34 (0.57, 3.22)
	>30		71	51	71.8	
Genotype	B,C	4	83	44	53.0	2.07 (0.95, 4.54)
	Non-B,C		51	18	35.3	
	B,C	8	82	53	64.6	1.00 (0.45, 2.20)
	Non-B,C		51	33	64.7	
	B,C	16	81	65	80.2	1.15 (0.43, 2.94)
	Non-B,C		50	39	78.0	
	B,C	28	82	67	81.7	1.23 (0.46, 3.18)
	Non-B,C		51	40	78.4	
	B,C	44	80	65	81.3	1.25 (0.47, 3.27)
	Non-B,C		49	38	77.6	
	B,C	48	82	64	78.0	1.63 (0.68, 3.85)
	Non-B,C		51	35	68.6	

All lower limits of the 95% confidence intervals in Table 21 and Table 22 are smaller than 1, except for Race at Week 28 (Table 21) and Week 4 (Table 22), which were both 1.1. This is in concordance with logistic regression analysis resulting in no statistically significant associations between the four covariates and viral load. Therefore, the virological responses at Weeks 4, 8, 16, 28 and 44 do not appear to be correlated with Race, Sex, Age, and HBV Genotype.

Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Odds Ratio (OR) Calculations for Week 48 Responses to Therapy with Respect to Viral Response at Various Times on Treatment in an HBeAg+ Population

The PPV, NPV, and OR for histological, biochemical and HBeAg loss responses with respect to HBV viral load were calculated for the HBeAg+ patients.

Week 48 Response to Therapy Definitions

For each patient, the following three responses were measured at various times on treatment: Histological, Biochemical and HBeAg Loss.

The three above responses were defined as positive at week 48:

- Positive Histological response — improvement of histological status at Week 48 by at least 2 units of the Knodell necro-inflammatory score without deterioration of the fibrosis score compared to the histological status at baseline
- Positive Biochemical response — normalization of ALT test result at Week 48 compared to the biochemical status at the baseline
- Positive HBeAg Loss response — HBeAg undetectable at week 48.

Statistical analysis was performed to evaluate whether there is an association between each of the above Week 48 positive responses and a positive viral load response (defined as HBV DNA < 2000 IU/mL or ≥ 2 $\log_{10}$ decrease from screening) at Weeks 4, 8, 16, 28 or 44 on treatment. Conversely, statistical analysis was performed to evaluate whether there is an association between each of the above Week 48 negative responses and a negative viral load response (defined as HBV DNA ≥ 2000 IU/mL or < 2 $\log_{10}$ decrease from screening) at Weeks 4, 8, 16, 28 or 44 on treatments. Based on the information in Table 23 and Table 24, the viral response at Weeks 4, 8, 16, 28 and 44 is informative for predicting various responses at Week 48 (i.e., lower bound of the 95% Confidence Interval (CI) of OR exceeding 1). Table 23 and Table 24 illustrate that for the HBeAg+ population, the highest PPVs of the COBAS® TaqMan® HBV Test take place with individual responses between 53.8% and 85.7%, while the highest NPVs of the COBAS® TaqMan® HBV Test take place with the combination of all three responses being between 81.9% and 96.2%. This data indicates that between 81.9% and 96.2% of the HBeAg+ patients with negative early viral response (≥ 2000 IU/mL) are not expected to achieve all three responses to treatment at week 48 of treatment.

Table 23
Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for Three Individual Responses at Week 48 of Treatment Predicted by an Early Viral Response (< 2000 IU/mL) in HBeAg+ Patients

Week of Viral Response	Week 48 Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
4	Histology	36.4 (44/121)	66.7 (4/6)	1.14 (0.16, 13.10)
	Biochemical	45.6 (57/125)	71.4 (5/7)	2.10 (0.33, 22.67)
	HBeAg Loss	73.0 (89/122)	71.4 (5/7)	6.74 (1.02, 72.78)
8	Histology	36.6 (41/112)	71.4 (10/14)	1.44 (0.38, 6.69)
	Biochemical	47.0 (55/117)	73.3 (11/15)	2.44 (0.67, 11.05)
	HBeAg Loss	75.4 (86/114)	66.7 (10/15)	6.14 (1.71, 24.52)
16	Histology	39.4 (39/99)	76.0 (19/25)	2.06 (0.71, 6.83)
	Biochemical	51.0 (53/104)	80.8 (21/26)	4.36 (1.44, 15.79)
	HBeAg Loss	76.2 (77/101)	53.8 (14/26)	3.74 (1.38, 10.11)
28	Histology	41.6 (37/89)	78.4 (29/37)	2.58 (1.00, 7.24)
	Biochemical	54.7 (52/95)	81.1 (30/37)	5.18 (1.96, 15.21)
	HBeAg Loss	85.7 (78/91)	65.8 (25/38)	11.54 (4.35, 31.03)
44	Histology	43.8 (32/73)	75.5 (37/49)	2.41 (1.02, 5.88)
	Biochemical	62.8 (49/78)	85.7 (42/49)	10.14 (3.79, 29.75)
	HBeAg Loss	93.2 (69/74)	66.0 (33/50)	26.79 (8.41, 97.69)

[1] *The denominator is the number of patients predicted not to have an individual response to treatment at week 48 based on the lack of an early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of an early viral response of < 2000 IU/mL.*

[2] *The denominator is the number of patients predicted to have an individual week 48 response to treatment based on an early viral response of < 2000 IU/mL; the numerator is the number of patients who had a respective week 48 response to treatment among the patients with early viral response of <2000 IU/mL.*

Table 24
Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for a Combination of All Three Responses at Week 48 on of Treatment Predicted by an Early Viral Response (< 2000 IU/mL) for an HBeAg+ Population) Predicted by Early Viral Response (< 2000 IU/mL)

Week of Viral Response	NPV % (Proportion[1])	PPV % (Proportion[2])	OR (95% CI)
4	81.9 (104/127)	16.7 (1/6)	0.90 (0.02, 8.66)
8	83.9 (99/118)	35.7 (5/14)	2.89 (0.68, 10.85)
16	85.7 (90/105)	36.0 (9/25)	3.38 (1.09, 9.91)
28	91.6 (87/95)	43.2 (16/37)	8.29 (2.84, 25.10)
44	96.2 (76/79)	42.9 (21/49)	19.00 (4.98, 104.34)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response of < 2000 IU/mL.*

[2] *The denominator is the number of patients predicted to have week 48 response to treatment based on early viral response of < 2000 IU/mL; the numerator is the number of patients who had all three responses to treatment at week 48 among the patients with early viral response of < 2000 IU/mL.*

With the viral response defined as ≥ 2 $\log_{10}$ decrease in value from the Screening viral load result, for the HBeAg+ population, the PPVs of COBAS® TaqMan® HBV Test are larger for the individual responses. (*see* Table 25) 36.3% to 70.5%, while the NPVs are the largest for the combination of all three responses, ranging from 87.5% to 96.3% (*see* Table 26).

Table 25
Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for Three Individual Responses at Week 48 on Treatment Predicted by Early Viral Response (≥2 $\log_{10}$ Decrease from Screening) in HBeAg+ Patients

Week of Viral Response	Week 48 Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
4	Histology	43.1 (28/65)	70.5 (43/61)	1.81 (0.81, 4.05)
	Biochemical	58.6 (41/70)	70.5 (43/61)	3.38 (1.54, 7.48)
	HBeAg Loss	82.4 (56/68)	43.3 (26/60)	3.57 (1.49, 8.77)
8	Histology	47.6 (20/42)	69.9 (58/83)	2.11 (0.91, 4.85)
	Biochemical	60.9 (28/46)	63.5 (54/85)	2.71 (1.22, 6.08)
	HBeAg Loss	89.1 (41/46)	40.2 (33/82)	5.52 (1.88, 19.55)
16	Histology	43.5 (10/23)	65.0 (65/100)	1.43 (0.50, 3.94)
	Biochemical	63.0 (17/27)	59.8 (61/102)	2.53 (0.97, 6.80)
	HBeAg Loss	96.3 (26/27)	37.4 (37/99)	15.52 (2.31, 652.99)
28	Histology	47.6 (10/21)	66.3 (69/104)	1.79 (0.61, 5.14)
	Biochemical	76.0 (19/25)	62.3 (66/106)	5.23 (1.79, 17.15)
	HBeAg Loss	96.2 (25/26)	36.3 (37/102)	14.23 (2.12, 599.78)
44	Histology	45.5 (10/22)	65.7 (65/99)	1.59 (0.55, 4.48)
	Biochemical	76.9 (20/26)	64.0 (64/100)	5.93 (2.03, 19.45)
	HBeAg Loss	96.0 (24/25)	37.8 (37/98)	14.56 (2.15, 614.51)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response (≥2 $\log_{10}$ IU/mL decrease); the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response (≥ 2 $\log_{10}$ IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 48 response to treatment based on early viral response (≥ 2 $\log_{10}$ IU/mL decrease); the numerator is the number of patients who had respective week 48 response to treatment among the patients with early viral response (≥2 $\log_{10}$ IU/mL decrease).*

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Table 26
Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for a Combination of All Three Responses at Week 48 on of Treatment Predicted by an Early Viral Response (≥2 log_{10} Decrease from Screening Viral Load) for HBeAg+ population Predicted by Early Viral Response (≥2 log_{10} Decrease from Screening)

Week of Viral Response	NPV % (Proportion[1])	PPV % (Proportion[2])	OR (95% CI)
4	87.5 (63/72)	25.0 (15/60)	2.33 (0.86, 6.58)
8	93.6 (44/47)	25.0 (21/84)	4.89 (1.32, 26.88)
16	96.3 (26/27)	22.5 (23/102)	7.57 (1.10, 323.62)
28	96.2 (25/26)	21.9 (23/105)	7.01 (1.02, 300.23)
44	96.2 (25/26)	22.8 (23/101)	7.37 (1.07, 315.62)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response (≥2 log_{10} IU/mL decrease); the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response (≥2 log_{10} IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 48 response to treatment based on early viral response (≥2 log_{10} IU/mL decrease); the numerator is the number of patients who had all three week 48 responses to treatment among the patients with early viral response (≥2 log_{10} IU/mL decrease).*

HBeAg loss is the most favorable endpoint in HBeAg+ patients receiving anti-viral therapy, since this allows possible safe discontinuation of therapy with a low likelihood of off-treatment relapse[43,44]. The results demonstrate that HBV DNA measurements of less than 2000 IU/mL or more than a 2 log_{10} decrease from a Screening HBV viral load at Weeks 4, 8, 16, 28 and 44 are associated with HBeAg loss at Week 48. A NPV of not achieving a 2 log_{10} decrease from a Screening HBV viral load result at an early stage of treatment appears medically associated with not achieving HBeAg loss at Week 48.

Clinical Performance of the COBAS® TaqMan® HBV Test in HBeAg- Patients on Therapy

Table 27 demonstrates the efficacy, based on HBV viral load testing, of treating HBeAg- patients with 10 mg Adefovir Dipivoxil compared to placebo. For example, at week 44 on treatment, 46.2% of HBeAg- patients on medication vs. 0% on placebo had achieved very low viral loads below 100 IU/mL. Furthermore, 74% of patients on medication vs. 2 % on placebo had achieved viral loads measuring below 400 IU/mL. Only 1.1% of patients on medication vs. 34% on placebo had a viral load exceeding 106 IU/mL.

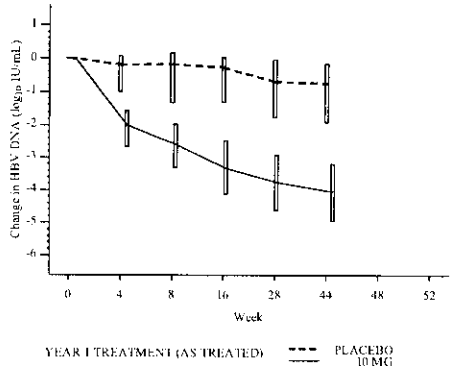
Table 27
Distribution of the HBV Viral Load at Week 44 on Treatment for HBeAg- Patients

Viral Load (IU/mL)	Adefovir Dipivoxil			Placebo		
	N	%	Cumul %	N	%	Cumul %
TND*	5	5.4	5.4	0	0.0	0.0
< 10	22	23.7	29.0	0	0.0	0.0
10 - < 100	16	17.2	46.2	0	0.0	0.0
100 - <400	26	28.0	74.2	1	2.0	2.0
400 - $<10^3$	6	6.5	80.6	2	4.0	6.0
10^3 - $<10^4$	6	6.5	87.1	8	16.0	22.0
10^4 - $<10^5$	5	5.4	92.5	12	24.0	46.0
10^5 - $<10^6$	6	6.5	98.9	10	20.0	66.0
10^6 - $<10^8$	1	1.1	100.0	17	34.0	100.0
Total	93			50		

**TND = HBV DNA not detected.*

The graph in Figure 13 shows the curves drawn through the median viral loads at various times on treatment, along with the inter-quartile ranges, for the HBeAg- patients both on medication (lower curve) and on placebo (upper curve).

Figure 13
Median and Inter-Quartile Range of Change in HBV DNA from Screening: HBeAg- Population



A useful tool for a clinician in assessing the effect of antiviral treatment for HBeAg- patients is watching for the HBV viral load increase of more than 1 log_{10} after reaching a nadir. Table 28 summarizes the data for the 90 HBeAg- patients on adefovir dipivoxil treatment.

Table 28
Distribution of the HBeAg- Patients by Week on Treatment and the Viral Load at Which the Nadir was Reached

Nadir Viral Load (IU/mL)	Number (%) of Patients With the Nadir Viral Load Achieved by Week					Total By Viral Load	Cumulative By Viral Load
	4	8	16	28	44		
TND*	1 (1.1)	2 (2.2)	4 (4.3)	1 (1.1)	3 (3.2)	11 (11.8)	11 (11.8)
< 10	0	0	3 (3.2)	3 (3.2)	12 (12.9)	18 (19.4)	29 (31.2)
10 – < 100	0	1 (1.1)	0	11 (11.8)	12 (12.9)	24 (25.8)	53 (57.00)
100 – < 10^3	0	0	0	8 (8.6)	15 (16.1)	23 (24.7)	76 (81.7)
10^3 – < 10^4	1 (1.1)	0	0	0	6 (6.5)	7 (7.5)	83 (89.2)
10^4 – < 10^5	0	2 (2.2)	1 (1.1)	0	2 (2.2)	5 (5.4)	88 (94.6)
10^5 – < 10^6	0	0	1 (1.1)	0	3 (3.2)	4 (4.3)	92 (98.9)
≥ 10^6	0	0	0	1 (1.1)	0	1 (1.1)	93 (100)
Total By Week	2 (2.2)	5 (5.4)	9 (9.7)	24 (25.8)	53 (57.00)		
Cumulative By Week	2 (2.2)	7 (7.6)	16 (17.3)	40 (43.1)	93 (100)		

* *TND = HBV DNA not detected.*

In this study, 43% (40/90) of the patients reached a nadir in viral load by week 28 on treatment. Out of those, seven patients had more than 1 log_{10} increase in viral load by week 44 after achieving the nadir, which is 7.5% (7/93) of the total number of patients on treatment and 17.5% (7/40) of the patients who achieved a nadir.

Table 29 summarizes the results of analysis of association of the responses to treatment at week 44 and the covariates. With all lower limits of the 95% Confidence Intervals being smaller than 1 (i.e., between 0.00 and 0.52), there are no statistically significant associations.

Table 29
Association Between Responses to Treatment at Week 44 and Baseline Covariates for HBeAg- Patients

Response to Treatment	Covariate	Category	N	Number of Patients With Response	Proportion (%) of Patients With Response	Unadjusted Odds Ratio (95% CI)
Histological	Race	Asian	28	16	57.1	0.47 (0.16, 1.35)
		Other	58	43	74.1	
	Sex	Male	73	52	71.2	2.12 (0.52, 8.31)
		Female	13	7	53.8	
	Age	≤ 30	8	6	75.0	1.42 (0.23, 15.24)
		>30	78	53	67.9	
	Genotype	B,C	28	16	57.1	0.47 (0.16, 1.35)
		Non-B,C	58	43	74.1	
Biochemical	Race	Asian	30	18	60.0	0.75 (0.28, 2.05)
		Other	63	42	66.7	
	Sex	Male	79	47	59.5	0.11 (0.00, 0.84)
		Female	14	13	92.9	
	Age	≤ 30	8	5	62.5	0.91 (0.16, 6.26)
		>30	85	55	64.7	
	Genotype	B,C	30	18	60.0	0.75 (0.28, 2.05)
		Non-B,C	63	42	66.7	

Additional data analysis of the HBeAg- population to demonstrate clinical performance of the COBAS® TaqMan® HBV Test was done using two different definitions of the early virological response to treatment: (1) HBV viral load < 2000 IU/mL (or approximately 10^4 cp/mL)[44], (2) a decrease in serum HBV DNA from initial Screening viral load value by ≥ 2 $\log_{10}$.[44]

The statistical significance of the associations of the Race, Sex, Age and Genotype covariates with the viral response was studied. The logistic regression analysis of viral response as a function of the covariates showed no statistical significance of such associations for either definition of the viral response. In addition, odds ratios plus their exact 95% confidence intervals were calculated for both definitions of the viral response and summarized in Table 30 and Table 31.

Table 30
Odds Ratios for the Association Between Viral Response (<2000 IU/mL) and Covariates, by Week, for an HBeAg- Population

Covariate	Category	Week	N	Number Below 2000 IU/mL	Proportion (%) Below 2000 IU/mL	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	30	11	36.7	1.70 (0.59, 4.75)
	Other		63	16	25.4	
	Asian	8	28	16	57.1	1.78 (0.66, 4.84)
	Other		63	27	42.9	
	Asian	16	30	22	73.3	1.69 (0.60, 5.10)
	Other		63	39	61.9	
	Asian	28	29	22	75.9	1.26 (0.42, 4.10)
	Other		63	45	71.4	
	Asian	44	30	26	86.7	1.69 (0.46, 7.79)
	Other		63	50	79.4	
Sex	Male	4	79	23	29.1	1.03 (0.26, 4.95)
	Female		14	4	28.6	
	Male	8	78	38	48.7	1.52 (0.40, 6.43)
	Female		13	5	38.5	
	Male	16	79	51	64.6	0.73 (0.15, 2.84)
	Female		14	10	71.4	
	Male	28	78	57	73.1	1.09 (0.22, 4.30)
	Female		14	10	71.4	
	Male	44	79	64	81.0	0.71 (0.07, 3.76)
	Female		14	12	85.7	
Age	≤ 30	4	8	2	25.0	0.80 (0.07, 4.89)
	>30		85	25	29.4	
	≤ 30	8	8	3	37.5	0.65 (0.09, 3.58)
	>30		83	40	48.2	
	≤ 30	16	8	4	50.0	0.49 (0.09, 2.87)
	>30		85	57	67.1	
	≤ 30	28	8	5	62.5	0.59 (0.11, 4.15)
	>30		84	62	73.8	
	≤ 30	44	8	6	75.0	0.64 (0.10, 7.15)
	>30		85	70	82.4	
Genotype	B,C	4	30	11	36.7	1.70 (0.59, 4.75)
	Non-B,C		63	16	25.4	
	B,C	8	28	16	57.1	1.78 (0.66, 4.84)
	Non-B,C		63	27	42.9	
	B,C	16	30	22	73.3	1.69 (0.60, 5.10)
	Non-B,C		63	39	61.9	
	B,C	28	29	22	75.9	1.26 (0.42, 4.10)
	Non-B,C		63	45	71.4	
	B,C	44	30	26	86.7	1.69 (0.46, 7.79)
	Non-B,C		63	50	79.4	

Table 31
Odds Ratios for the Association Between Viral Response
(≥2 log_{10} Decrease From Initial Screening Result) and Covariates, by Week, for an HBeAg- Population

Covariate	Category	Week	N	Number With ≥ 2 log_{10} Decrease	Proportion (%) With ≥ 2 log_{10} Decrease	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	30	15	50.0	1.07 (0.41, 2.79)
	Other		62	30	48.4	
	Asian	8	28	20	71.4	0.80 (0.27, 2.54)
	Other		62	47	75.8	
	Asian	16	30	26	86.7	1.40 (0.37, 6.61)
	Other		62	51	82.3	
	Asian	28	29	27	93.1	2.60 (0.50, 25.81)
	Other		62	52	83.9	
	Asian	44	30	28	93.3	2.38 (0.44, 23.90)
	Other		62	53	85.5	
Sex	Male	4	78	37	47.4	0.68 (0.18, 2.47)
	Female		14	8	57.1	
	Male	8	77	56	72.7	0.48 (0.05, 2.53)
	Female		13	11	84.6	
	Male	16	78	64	82.1	0.35 (0.01, 2.76)
	Female		14	13	92.9	
	Male	28	77	65	84.4	0.00 (0.00, 1.49)
	Female		14	14	100.0	
	Male	44	78	67	85.9	0.00 (0.00, 1.69)
	Female		14	14	100.0	
Age	≤ 30	4	8	2	25.0	0.32 (0.03, 1.93)
	>30		84	43	51.2	
	≤ 30	8	8	6	75.0	1.03 (0.17, 11.22)
	>30		82	61	74.4	
	≤ 30	16	8	6	75.0	0.55 (0.09, 6.19)
	>30		84	71	84.5	
	≤ 30	28	8	6	75.0	0.41 (0.06, 4.76)
	>30		83	73	88.0	
	≤ 30	44	8	6	75.0	0.36 (0.05, 4.23)
	>30		84	75	89.3	
Genotype	B,C	4	30	15	50.0	1.07 (0.41, 2.79)
	Non-B,C		62	30	48.4	
	B,C	8	28	20	71.4	0.80 (0.27, 2.54)
	Non-B,C		62	47	75.8	
	B,C	16	30	26	86.7	1.40 (0.37, 6.61)
	Non-B,C		62	51	82.3	
	B,C	28	29	27	93.1	2.60 (0.50, 25.81)
	Non-B,C		62	52	83.9	
	B,C	44	30	28	93.3	2.38 (0.44, 23.90)
	Non-B,C		62	53	85.5	

All lower limits of the 95% confidence intervals in Table 30 and Table 31 are smaller than 1. This means that there are no statistically significant associations between the four covariates and the viral load, and, therefore, the virological responses at Weeks 4, 8, 16, 28 and 44 do not appear to be correlated with race, sex, age, and HBV genotype.

Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Odds Ratio (OR) Calculations for Week 44 Responses to Therapy with Respect to Viral Response at Various Times on Treatment (HBeAg- Patients)

The PPV, NPV, and OR for the histological and biochemical responses with respect to HBV viral load determined using the COBAS® TaqMan® HBV Test were calculated for the HBeAg- patients.

Week 44 Response to Therapy Definitions for HBeAg- Patients

For each patient, the following two responses were measured at various times on treatment: Histological and Biochemical.

The two responses were defined as positive at week 44:

- Positive Histological response — improvement of histological status at Week 44 by at least 2 units of the Knodell necro-inflammatory score without deterioration of the fibrosis score compare to the histological status at baseline
- Positive Biochemical response — normalization of ALT test result at Week 44 compared to the biochemical status at the baseline

Statistical analysis was performed to evaluate whether there is an association between each of the above Week 44 positive responses and a positive viral load response (defined as HBV DNA < 2000 IU/mL or ≥ 2 $\log_{10}$ decrease from screening) at Weeks 4, 8, 16 or 28 on treatment. Conversely, statistical analysis was performed to evaluate whether there is an association between each of the above Week 44 negative responses and a negative viral load response (defined as HBV DNA ≥ 2000 IU/mL or < 2 $\log_{10}$ decrease from screening) at weeks 4, 8, 16 or 28 on treatment.

Based on the information in Table 32 and Table 33, the viral response (HBV DNA < 2000 IU/mL) at Weeks 4, 8, 16 and 28 is not informative for predicting various Week 44 responses for HBeAg- patients with the data available (the lower limits of the 95% CIs are between 0.11 and 0.89, below 1, for various responses at various times on treatment).

Table 32
Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for Two Individual Responses at Week 44 of Treatment Predicted by Early Viral Response (< 2000 IU/mL) in HBeAg- Patients

Week of Viral Response	Week 44 Response	NPV (Proportion[1])	PPV (Proportion[2])	OR (95% CI)
4	Histology	25.0 (16/64)	50.0 (11/22)	0.33 (0.11, 1.04)
	Biochemical	31.8 (21/66)	55.6 (15/27)	0.58 (0.21, 1.63)
8	Histology	23.4 (11/47)	57.9 (22/38)	0.42 (0.15, 1.18)
	Biochemical	35.4 (17/48)	65.1 (28/43)	1.02 (0.40, 2.66)
16	Histology	30.0 (9/30)	67.9 (38/56)	0.90 (0.30, 2.59)
	Biochemical	43.8 (14/32)	68.9 (42/61)	1.72 (0.64, 4.54)
28	Histology	33.3 (8/24)	68.9 (42/61)	1.11 (0.35, 3.33)
	Biochemical	52.0 (13/25)	70.1 (47/67)	2.55 (0.89, 7.26)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 44 based on the lack of early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response of < 2000 IU/mL.*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response of < 2000 IU/mL; the numerator is the number of patients who had respective week 48 response to treatment among the patients with early viral response of < 2000 IU/mL.*

Table 33

Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for Both Biochemical and Histological Responses at Week 44 of Treatment Predicted by Early Viral Response (< 2000 IU/mL) in HBeAg- Patients

Week of Viral Response	NPV (Proportion[1])	PPV (Proportion[2])	OR (95% CI)
4	49.2 (32/65)	29.2 (7/24)	0.40 (0.12, 1.19)
8	50.0 (24/48)	40.0 (16/40)	0.67 (0.26, 1.69)
16	62.5 (20/32)	49.1 (28/57)	1.61 (0.61, 4.32)
28	72.0 (18/25)	50.8 (32/63)	2.65 (0.89, 8.53)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 44 response (no histological or biochemical response) to treatment at week 44 among the patients with the lack of early viral response of < 2000 IU/mL.*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response of < 2000 IU/mL; the numerator is the number of patients who had both (histological and biochemical) responses to treatment at week 44 among the patients with early viral response of < 2000 IU/mL.*

Table 34 and Table 35 demonstrate that for HBeAg- patients, viral response (defined as ≥ 2 $\log_{10}$ decrease in value from the Screening viral load result) at weeks 8, 16 and 28 is informative for prediction of biochemical response at week 44 on treatment, and it is informative at weeks 16 and 28 for prediction of having both responses (histological and biochemical) at week 44 on treatment (i.e., lower limits of the 95% CIs exceed 1). The NPV for the biochemical response is from 73.3% at week 16 to 83.3% at week 28. The PPV is approximately 71% at both weeks 16 and 28.

Table 34
Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for Two Individual Responses at Week 44 of Treatment Predicted by Early Viral Response (≥ 2 $\log_{10}$ Decrease from Screening Viral Load) in HBeAg- Patients

Week of Viral Response	Week 44 Response	NPV (Proportion[1])	PPV (Proportion[2])	OR (95% CI)
4	Histology	28.3 (13/46)	64.1 (25/39)	0.70 (0.25, 1.94)
	Biochemical	44.7 (21/47)	73.3 (33/45)	2.22 (0.85, 5.90)
8	Histology	31.8 (7/22)	67.7 (42/62)	0.98 (0.29, 3.07)
	Biochemical	56.5 (13/23)	71.6 (48/67)	3.28 (1.10, 9.86)
16	Histology	30.8 (4/13)	68.1 (49/72)	0.95 (0.19, 3.85)
	Biochemical	73.3 (11/15)	71.4 (55/77)	6.88 (1.76, 32.07)
28	Histology	18.2 (2/11)	65.8 (48/73)	0.43 (0.04, 2.31)
	Biochemical	83.3 (10/12)	70.9 (56/79)	12.17 (2.27, 119.14)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 44 based on the lack of early viral response (≥ 2 $\log_{10}$ IU/mL decrease); the numerator is the number of patients who had no respective week 44 response to treatment among the patients with the lack of early viral response (≥ 2 $\log_{10}$ IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response (≥ 2 $\log_{10}$ IU/mL decrease); the numerator is the number of patients who had respective week 44 response to treatment among the patients with early viral response (≥ 2 $\log_{10}$ IU/mL decrease).*

Table 35

Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for Combination of Both Biochemical and Histological Responses at Week 44 of Treatment Predicted by an Early Viral Response (≥2 log_{10} Decrease from Screening Viral Load) in HBeAg- Patients

Week of Viral Response	NPV (Proportion[1])	PPV (Proportion[2])	Odds Ratio (95% CI)
4	59.6 (28/47)	48.8 (20/41)	1.40 (0.55, 3.56)
8	73.9 (17/23)	51.6 (33/64)	3.02 (0.96, 10.47)
16	86.7 (13/15)	50.7 (37/73)	6.68 (1.34, 63.95)
28	91.7 (11/12)	49.3 (37/75)	10.71 (1.40, 473.19)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 44 based on the lack of early viral response (≥ 2 log_{10} IU/mL decrease); the numerator is the number of patients who had no respective week 44 response (no histological or biochemical response) to treatment among the patients with the lack of early viral response (≥ 2 log_{10} IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response (≥ 2 log_{10} IU/mL decrease); the numerator is the number of patients who had both two responses to treatment at week 44 among the patients with early viral response (≥ 2 log_{10} IU/mL decrease).*

Statistical analysis of clinical data has shown that viral response to treatment measured with COBAS® TaqMan® HBV Test For Use With the High Pure System is informative for assessing the effect of treatment in patients with chronic hepatitis B.

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